AMRL-TR-76-66

DETERMINATION OF EFFECTS OF DESIGNATED POLLUTANTS ON PLANT SPECIES ANNUAL REPORT

STATEWIDE AIR POLLUTION RESEARCH CENTER
UNIVERSITY OF CALIFORNIA, RIVERSIDE, CA 92502

OCTOBER 1976

AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 80-33.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Anthony A. Thomas

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Aerospace Medical Research Laboratory

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Hydrogen Chloride (HCl)	Gas Generator	Plant Growth Stages
Hydrogen Fluoride (HF)	Aerosol Generator	Plant Nutrition
Aluminum Oxide Particles (Al_2O_3)	Ornamental Plants	Threshold Dosage

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The phytotoxic responses of selected plants to missile exhaust products were studied. The plants included but were not limited to ornamental, vegetable, and field crops found in the vicinity of Vandenberg Air Force Base and were grown in greenhouses equipped with evaporative coolers with activated charcoal air filters. The missile products investigated were hydrogen chloride and hydrogen fluoride gases and aluminum oxide aerosols, alone and in various combinations of toxicants. The gases were generated by the volatilization of acid liquids into a hot air stream and the aerosols were generated using nitrogen gas to carry the

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particles through a cylinder haying a reseryoir of particles. The gases were monitored in the exposure chambers by trapping and titrating known air samples. Most studies concentrated on HCl gas alone.

The nutrient level in which nasturtium and marigolds were raised affected the tolerance of the plant to the HCl treatment. Both species, when supplied with excess nutrients, could better tolerate the exposures to HCl gas.

Two varieties of tomato were compared in their susceptibility to HCl, HF, and HCl plus HF. Ace, a true line variety, seemed slightly more tolerant to damage than the hybrid variety 6718VF. The two varieties and barley were given single exposures of the above three pollutants at different stages during their development. Differences in sensitivity between these stages was slight. The single exposures do not seem to affect plant productivity greatly.

Threshold experiments verified that the data for short duration exposures fit a hyperbolic curve with lower concentrations of HCl necessary for injury at 20 minutes than at 5 minutes. The minimum concentration necessary for 20-minute exposures was 10 mg/m^3 for radish, a very sensitive host.

Aluminum oxide plus HCl may produce slightly more injury on marigolds than if HCl were the sole pollutant. More sensitive hosts are being sought to further test this finding.

Experiments where marigolds were exposed for short, weekly fumigations provide evidence that even low dosages alter the final biomass of the plant, although no injury was visible.

The research conducted this year forms the basis of continued investigations on assessing the impact of the designated pollutants on vegetation.

PREFACE

This is the first Annual Report of work performed under the Environmental Toxicology Research sponsored by Air Force Contract F-33615-76C-5005. The work under this portion of the contract covers the period from September 1, 1975, to June 30, 1976. This project is titled: "Determination of Effects of Designated Pollutants on Plant Species" and was conducted by members of the Statewide Air Pollution Research Center, University of California, Riverside. The study was designed to aid Air Force personnel to recognize and predict the phytotoxic responses of terrestrial plants to air pollutants released to the atmosphere by Air Force operations. The terrestrial plants include, but are not limited to, commercial crops grown in the vicinity of Vandenberg Air Force Base. The pollutants under consideration are hydrogen chloride and hydrogen fluoride gases and aluminum oxide aerosols, as well as combinations of these three.

The authors gratefully acknowledge S. Lerman for initiating these studies; R. J. Oshima for critical advice; M. J. Harris, P. McCool, L. A. Neher, L. Nolan, and T. R. Thomas for technical assistance in various phases of the project.

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I. BACKGROUND INFORMATION

Review of hydrogen chloride and chlorine effects on vegetation

Chlorine is an important plant constituent which occurs largely as soluble chloride compounds in water, soil solutions, and in the soil. Some crops are tolerant of chlorides while others are very sensitive. High chloride applied as a nutrient may cause physiological changes in plants and may interfere with carbohydrate metabolism.

Although small amounts of chlorine are essential for proper plant development, excessive amounts can be highly toxic. In some instances trade winds have carried fine spray from the oceans for considerable distances inland to produce deleterious effects and to cause significant increase in chloride content of plant tissues.

Leaf injury and elevated chloride levels in tissues are frequently found when crops such as citrus, grapes, and almonds are sprinkler irrigated with water containing excessive amounts of soluble chloride. In such irrigation practices, where foliage is wetted by the high chloride water, injury symptoms may appear in a few hours. Successive irrigations may also result in accumulation of chlorides. The critical level of chloride in leaf tissue of citrus, almonds, and grapes required to produce visible symptoms seems to be about 2% chlorine ion on a dry leaf weight basis. Application of sodium chloride to remove ice from highways or the use of calcium chloride as a dust palliative may result in chloride injury to vegetation growing nearby.

Chlorine and hydrogen chloride (HCl) are readily absorbed from polluted atmospheres by plant foliage. When absorbed slowly from atmospheres containing low concentrations, the resultant chlorides are additive to those absorbed through the root system. Apparently there is little if any deleterious effect on normal plants unless the threshold for injury is exceeded by accumulation at peripheral zones of the foliage where the chloride is deposited. However, high atmospheric concentrations of HCl or chlorine may result in lethal concentrations of acid formation by exceeding the buffering capacity of local zones of tissue. When this occurs, characteristic necrotic lesions develop or patterns of chlorotic tissue develop.

Under field conditions where accidental releases of very high concentrations of chlorine and of HCl have occurred for brief periods, severe defoliation of deciduous plants has been observed. Defoliation may occur within a few hours following the incident and frequently the fallen leaves display no visible symptoms of injury. Apparently the gases stimulate rapid development of the abscission zone without necrosis or pigment change.

The role of moisture deposits on leaves, whether as acid precipitation or rain, in enhancing chloride injury has been considered. It was generally assumed that solutes enter leaves almost exclusively through stomata. Passage of solutes such as chloride through cuticle is questioned. Whether substances pass through the epidermis or stomata, they may have to

pass through a layer of cuticle since it is reported (Jung and Wittwer, 1964) that such a layer commonly covers the inner walls of the leaf epidermis and the walls of mesophyll cells adjoining substomatal cavities. Characteristics of external and internal cuticle may play an important role in the differential susceptibility of various species and cultivars of plants. Yamada (1964) reported that cations and anions penetrated tomato fruit cuticle with no stomata as readily as they penetrated onion leaf cuticle with stomata. This suggests that chloride absorbed in surface moisture deposited on leaves may readily penetrate the cuticle irrespective of stomata.

Review of aluminum oxide effects on vegetation

Aluminum ions in the soil are highly toxic to plants in areas of acid soils (Foy, 1974). Vast areas of acid soils in South America and of acid subsoils in the Southeastern United States have limited productivity because of aluminum toxicity. It is highly improbable that deposits of aluminum oxide from missile exhaust would be sufficient to significantly increase the aluminum ion toxicity of soils, but the possibility of toxicity to aerial portions of the plant should be carefully considered.

Evidence of aluminum toxicity from deposits of particulates on the foliage has not come to the attention of researchers involved in this project but the possibility is receiving attention. With the appropriate combination of moisture, HCl gas, and microscopic aluminum particles on leaf surfaces, one can readily hypothesize that aluminum ions may penetrate the leaf cuticle in sufficient quantity to be phytotoxic.

Aluminum particles emitted simultaneously with HCl into humid atmospheres may serve as nuclei for an acid aerosol and effectively concentrate the HCl impinging on the surface of leaves. Investigations of combined effects of aluminum particles and HCl are essential to determine if HCl phytotoxicity is enhanced.

Hydrogen fluoride

Initially, it was indicated that at a future date, fluorine may be used in missile propellants. Consequently, investigations were initiated to study effects of HF under such conditions, but these studies are being delayed to concentrate attention on aluminum particles and HCl.

The studies included in this report were designated to determine the critical concentration of HCl and aluminum oxide in the atmosphere required to produce injury on selected plant species and cultivars during exposure for periods of less than 30 minutes. The effects of the two compounds are being studied individually and in combination. Response of plants from the time they emerge until maturity are being considered.

II. EQUIPMENT, MATERIALS, AND GENERAL EXPERIMENTAL PROCEDURES

A. Selection of plants

Ten species or varieties of plants were investigated during the period of this report. The source and rationale for the selection of these ornamental and garden plants are listed in Table 1. Many of these were continued from our earlier investigations (Lerman, 1975), having been originally chosen because of their occurrence or cultivation in the vicinity of Vandenberg Air Force Base.

B. Production of plants

Most plants were transplanted from trays or sown directly in 10 cm pots containing UC soil mix II. The pine trees were bought as seedlings and transplanted to 11 X 36 cm pots. A sand-peat mix was used for plants in some nutrition experiments. The plants were raised in greenhouses equipped with evaporative coolers with activated charcoal filters. Daily temperatures varied between 26 and 32 C and night temperatures ranged between 15 and 21 C.

C. Exposure of plant material to the pollutants

Exposure chambers

Three chambers similar to those described by Heck et al. (1968) are now in use. Two of these are softwalled, being constructed of wood frames covered with Mylar plastic sheeting. These chambers are used only for gaseous pollutants. The third chamber is similar to the other two, but is constructed of plexiglas. It has been modified, as previously described (Lerman, 1975), to allow exposure of plants to both aerosol and gas pollutants. The chambers are all located within the greenhouse where plants are grown. They use filtered greenhouse air and exhaust polluted air outside the greenhouse.

Equipment for generating and dispensing HCl and HF gases

During the period covered by this report, the system of bubbling moist air through aqueous acid solutions (Lerman, 1975) was replaced with an automatic syringe system. A Sage Model 351 syringe pump forces HCl or HF solution from a 10 ml plastic disposable syringe at a constant rate through a Teflon No. 21 needle into the base of a tee fitting. Here the acid joins a 12 liter per minute flow of pre-heated room air in which it volatilizes immediately. The acid-saturated air now flows into a 120 cm long, 7.5 mm I.D. Teflon tube. Heat tape around the Teflon tube keeps air at ca. 95 C as it enters the manifold where it mixes with intake air and is distributed around the chamber. The syringe rate and the acid concentration can be varied to change pollution concentration in the chamber.

Table 1. List of plants for the phytotoxicity study

	Plant	Source	Rationale for Selection
Aster	*Callistephus chinesis* var Early Bird White	Burpee[1]	Previous experience; grown at Lompoc
Barley	*Hordeum vulgare* var CM 67	Burpee	Grown at Lompoc between flower seed crops
Bean	*Phaseolus vulgaris* var Pinto	Burpee	Rapid growth, widespread field and garden cultivation, good for comparisons with other investigators
Citrus	*Citrus sinesis* var Troyer	UCR-PPD[2]	Special use in limited studies
Cornflower	*Centaurea cyanus* var Jubilee Gem	Burpee	Grown commercially at Lompoc
Nasturtium	*Tropaeolum majus* var Primrose yellow	Burpee	Grown commercially at Lompoc
Marigold	*Tagetes erecta* var Senator Dirkson	Burpee	Grown commercially at Lompoc
Pine (bishop)	*Pinus muricata*	Calif. Div. Forestry[3]	Unique to California coast near Lompoc and Vandenberg AFB
Tomato	*Lycopersicon esculentum* var Ace	Burpee	Widely distributed garden plant, true line variety
Tomato	*Lycopersicon esculentum* var 6718 VF	Peto[4]	Hybrid variety may be less resistant than true line
Radish	*Raphanus sativus* var Comet	Burpee	Rapid growth, widespread distribution in home gardens

[1]W. Atlee Burpee Co., Riverside, California

[2]Plant Pathology Department, University of California, Riverside, California; local seed trees

[3]California Division of Forestry, Davis, California

[4]Peto Seed Co., Saticoy, California

Using this dispensing system, calibration curves were derived for the two types of chambers available (see Figures 1 and 2). For HCl generation, Figure 1, the variability between replications was relatively small in either chamber; however, when plants were introduced the reduction in HCl concentration was greater in the softwalled than in the plexiglas chamber. This has not been a problem, since the concentration is adjusted for the specific chamber. In addition, each fumigation chamber was tested for uniform toxicant concentration by generating HCl or ozone gas and then taking samples from different parts of the chamber. Sensitive plants were also exposed and amount of injury was compared to chamber location. These methods ensured the homogeneity of gas concentration during experimental plant exposures. Figure 2 is the calibration curve for HF gas in the two chambers and shows a similar drop in toxicant concentration when plants are present.

Monitoring HCl gas in the chambers

HCl is presently being monitored as previously described; that is, a glass tube protrudes into the center of the chamber at about plant height. During fumigation, a known amount of chamber air is drawn through the tube and bubbled through a 0.1 N nitric acid solution. A Scientific Products Model 63111 wet test meter is placed after the pump and the bubbler to record the actual volume of air sampled, usually about 15 liters. The chloride in the solution is determined with an American Instruments Model 4-4433 automatic chloride titrator.

Monitoring HF gas in the chambers

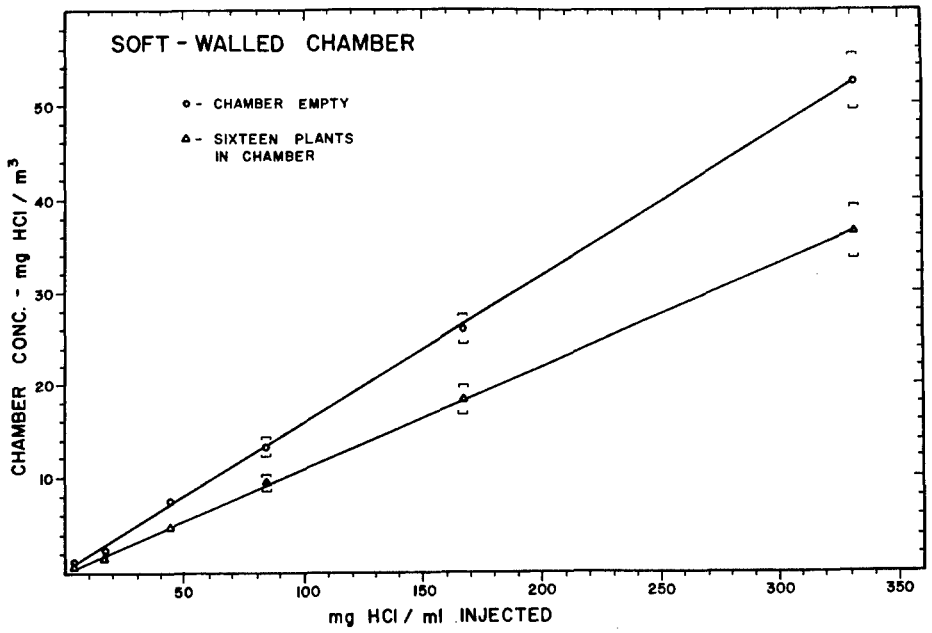
A given amount of air containing HF is drawn into the sampe tube and bubbled through TISAB (Total Ionic Strength Adjustment Buffer). The solution is analyzed for fluoride with an Orion Model 94-09 fluoride ion electrode.

Determination of chloride in foliage

As previously reported, harvested plant parts were oven-dried at 70 C for 24 hours, ground in a Wiley mill to pass through a 40 mesh screen, and eluted for 24 hours in a 0.1 N nitric acid solution containing polyvinyl alcohol and acetic acid. The chloride was then determined with the automatic chloride titrator.

Aluminum oxide (Al_2O_3) particle generation

A new generator was designed, tested, and calibrated that does not rely on mechanical agitation or require constant attention. This apparatus is easier to operate and is more dependable, since it does not need refilling after each exposure and can be rapidly adjusted.



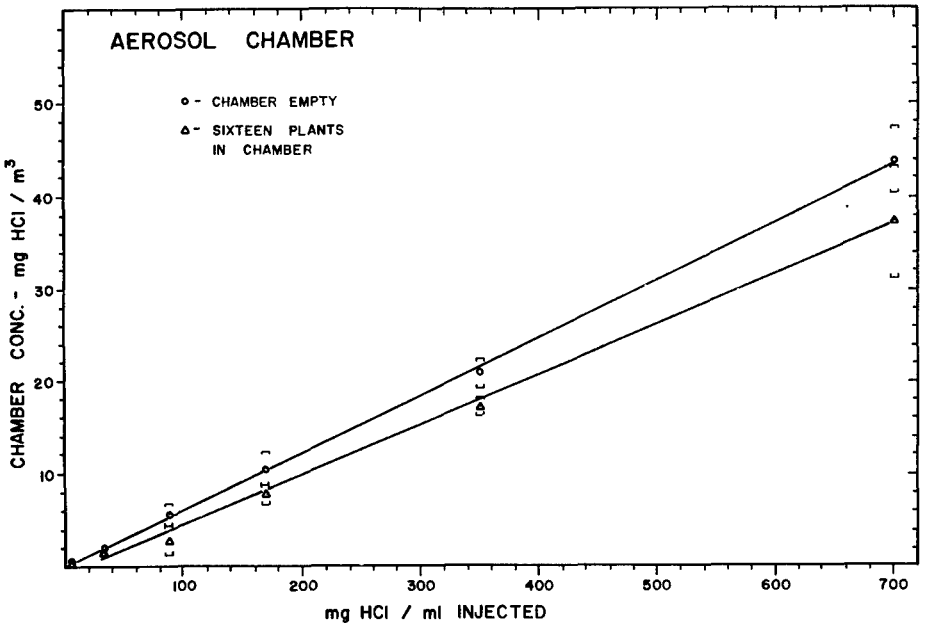
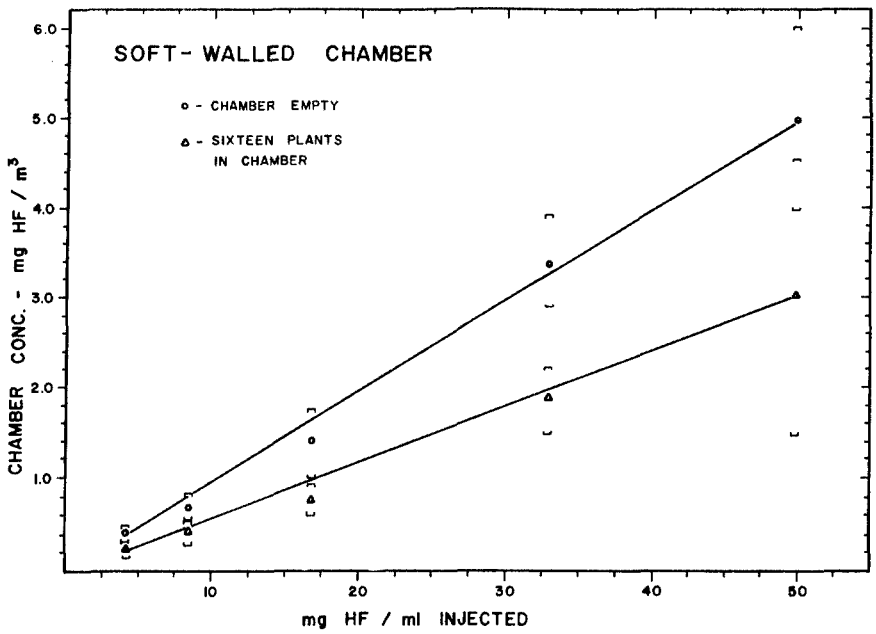


Figure 1. Calibration curves for hydrogen chloride gas (HCl) in two chamber types. Top: Chamber covered with Mylar film. Bottom: Plexiglas chamber. Six replications per point .



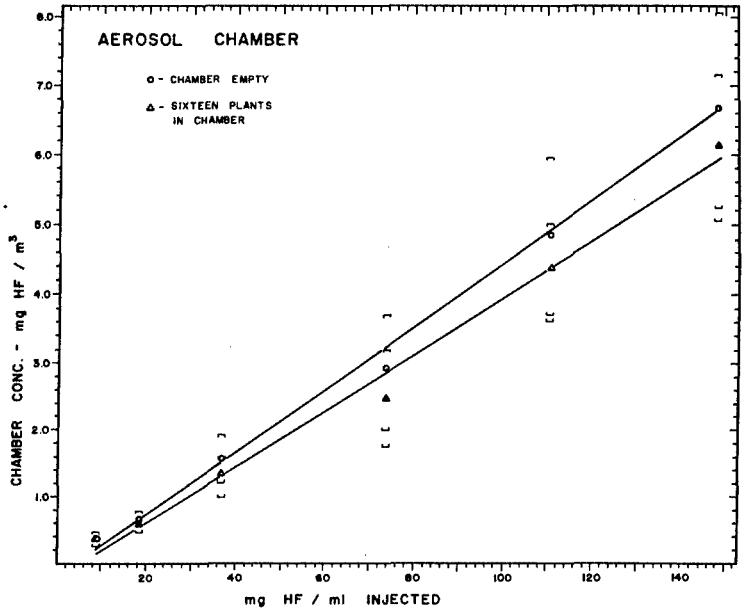


Figure 2. Calibration curves for hydrogen fluoride gas (HF) in two chamber types. Top: Chamber covered with Mylar film. Bottom: Plexiglass chamber. Six replications per point.

Figure 3 schematically illustrates the exposure chamber while Figure 4 is a diagram of the particle generator itself. Basically, a dry nitrogen gas is introduced at the base of the cylindrical generator to lift particles through its length and into the fumigating air stream. At higher nitrogen flow rates, more particles will be delivered to the air stream and so to the chamber and the plants. Figure 5 shows that the delivery is essentially linear. A high volume cascade impactor was used to sample the particles at two different flow rates to determine the particle size by weight as shown in Figure 6. Different flow rates do not greatly affect the size distribution and 90% of the total weight of the aluminum oxide delivered are particles equal to or smaller than 2.0 μ.

To establish that aluminum oxide particles were actually being delivered to and collecting on the exposed plants, various species were placed in the chamber and exposed to 9 mg Al_2O_3/m^3 plus 9 mg HCl/m^3 for 20 minutes. Pieces of leaf tissue were observed at 500 x magnification using a Leitz microscope equipped with incident illumination. Both the upper and lower leaf surfaces were inspected and the results are presented in Table 2. Apparently leaf structure and orientation have much to do with particle collection. Upright or thin leaves do not present a satisfactory collection surface for the dust.

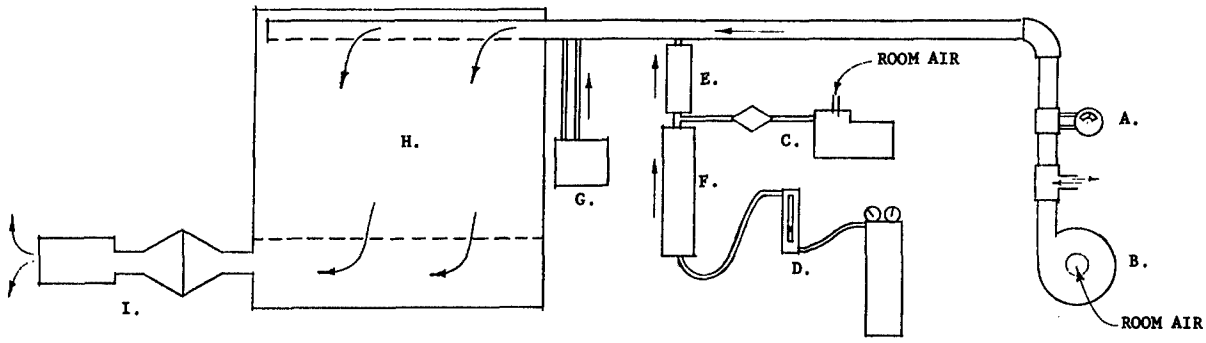
The submicron aluminum oxide particles were purchased from Research Organic/Inorganic Chemical Corporation, Belleville, New Jersey, and were characterized in the last report (Lerman, 1975).

Table 2. Light microscopy detection of aluminum oxide on leaf surface of plants

Plant	Leaf Surface	
	Upper	Lower
Barley	Trace	None seen
Bean	Heavy, even	None seen
Citrus	Sparse, even	None seen
Nasturtium	Sparce in center; heavy near edge	None in center; some edges had deposits
Pine	Trace	Not observed
Radish	Trace, mainly on trichomes	None seen
Tomato	Heavy, in uneven agglomerates	None seen

Exposure procedures

Plants of the same age were usually fumigated at the same time in groups of 12 or 16. A rack was constructed of a wooden frame and wire



A. Restricted orifice type flowmeter
B. Input blower
C. Filtered air compressor
D. Flowmetered Nitrogen source
E. Krypton radiation source (optional)
F. Particle generator
G. Gas generation source
H. Plant exposure chamber
I. Filtered Hi-Vol exhaust

Figure 3. Schematic diagram of gas-aerosol exposure chamber.

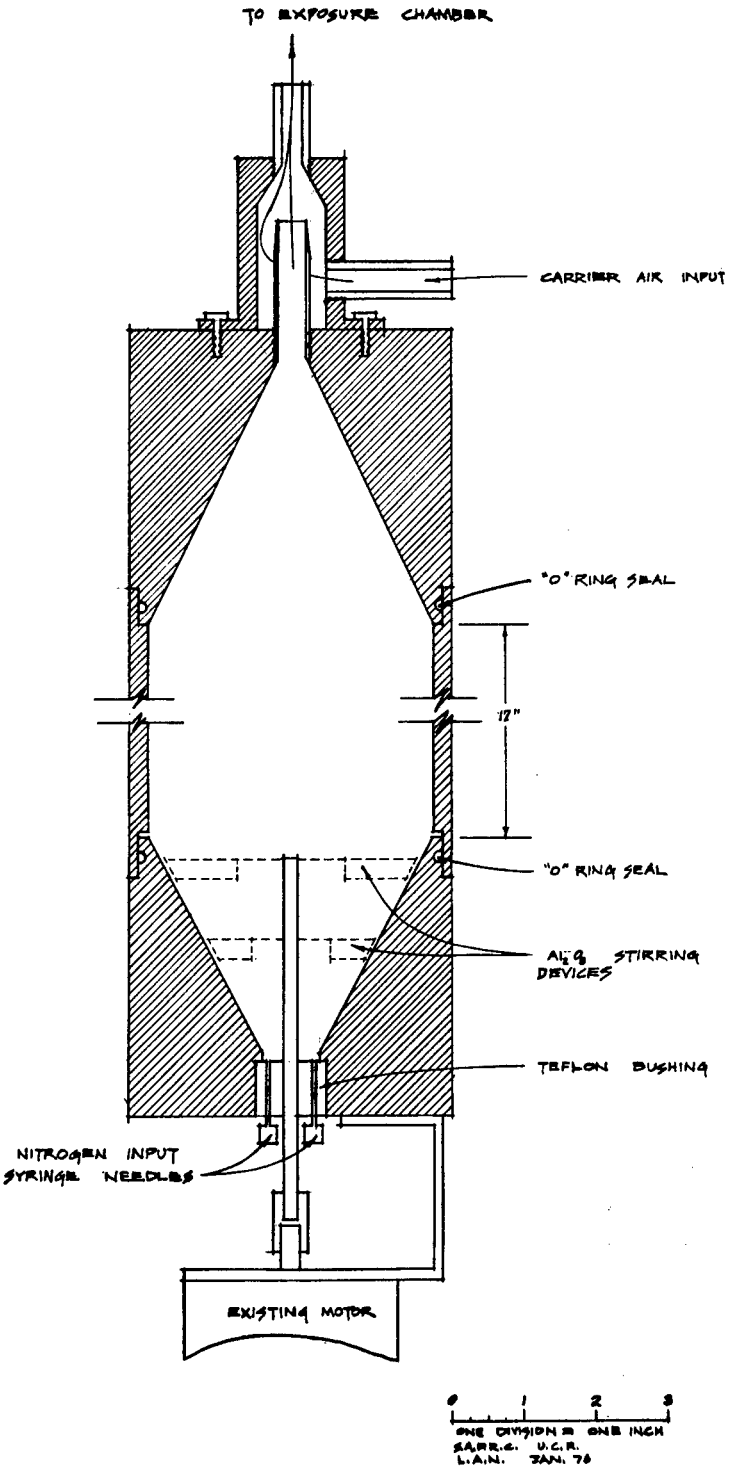


Figure 4. Aluminum oxide particulate generator machined from aluminum stock.

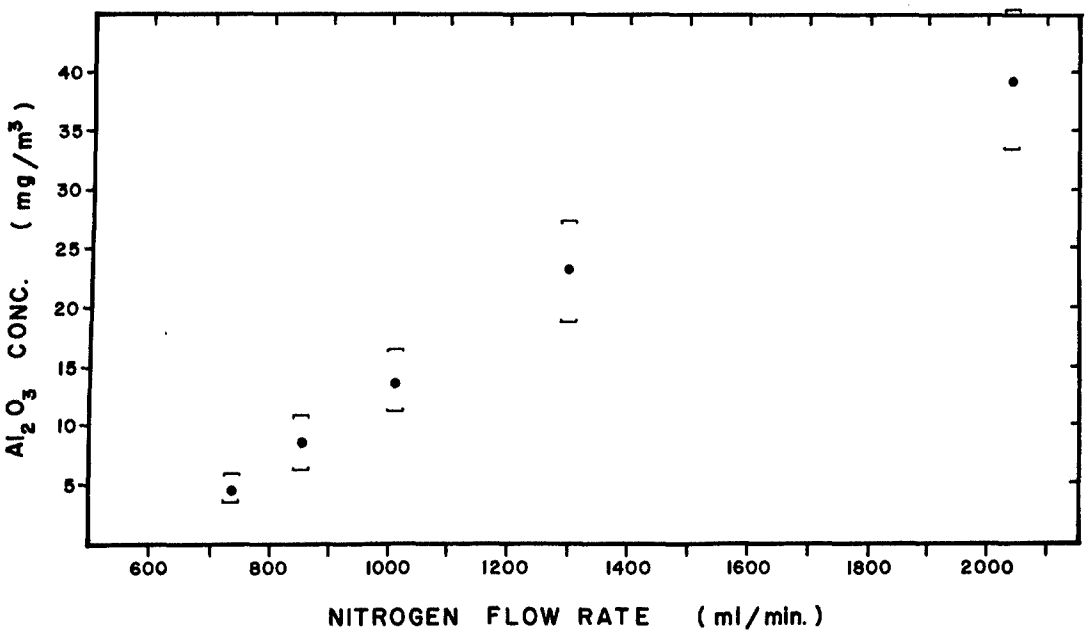


Figure 5. Particle generator calibration: Nitrogen flow rate for aluminum oxide delivery. Brackets refer to one standard deviation around particle concentration means (o).

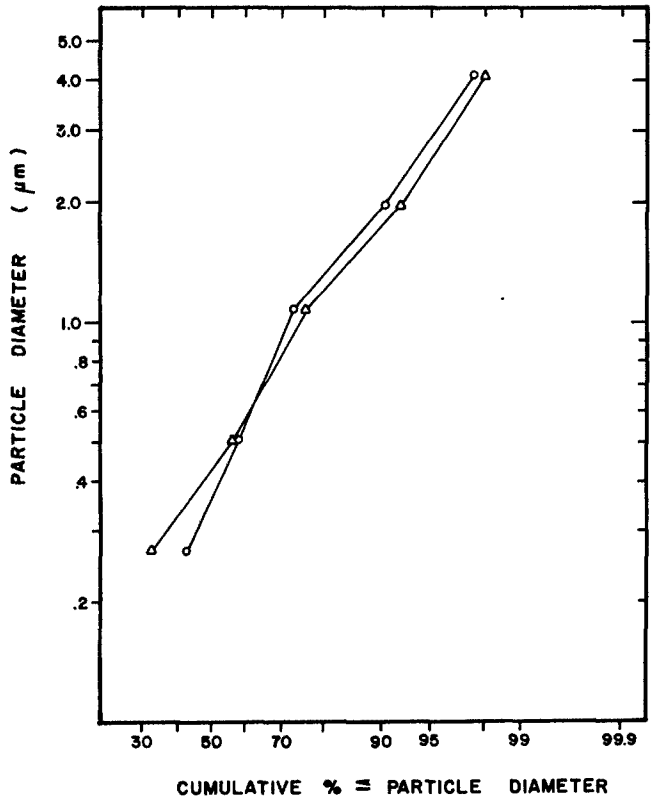


Figure 6. Particle generator calibration: Particle size distribution by mass at two delivery rates. Δ = 1000 ml N_2/min., 0 = 2000 ml N_2/min.

mesh top containing 12 evenly spaced holes, each of which accepted a four-inch pot. This rack enabled rapid manipulation of the plants in the soft-walled exposure chambers. These chambers had counterweighted front doors for easy accessibility. For each experiment, control plants were maintained under the same growing conditions, but were not placed in any chamber. The temperatures ranged from 24 to 35 C and relative humidity measured between 50 and 70%. A glass thermometer inside the chamber provided temperature measurement and a sling psychrometer was useful for determining relative humidity. In some cases, the relative humidity was measured with wet and dry bulb thermocouples placed in the base or exhaust of the chamber and recorded on a Honeywell Model 153X-67 recorder. Sunlight in the vicinity of the chamber was measured on a YSI Model 65 radiometer and light intensities were generally greater than 3×10^4 ergs/cm^2-sec.

Plants in these experiments were exposed for periods of 30 minutes or less, usually 5, 10, 15, or 20 minutes. These relatively short times were emphasized because the rocket emission cloud would not be expected to persist for much longer.

Evaluation of phytotoxic response

Following exposure, the plants were removed from the chamber and remained in the greenhouse for 24 to 48 hours before evaluation of any visible response. In most cases data recorded included number of plants damaged per fumigation, number of leaves damaged per plant, and a rough estimate of percent leaf damage (0-25%, 26-50%, 51-75%, over 75%). Where necessary or desirable, symptoms were described and photographic records made. In addition, some experiments extended in time either with additional fumigations or with prolonged observations. For these long-term experiments, height data were recorded regularly and plants were usually harvested at the end to obtain leaf, stem, and root dry weights, fruit or seed yields and, sometimes, the chlorine ion concentration of various tissues.

III. PHYTOTOXIC RESPONSES

A. Effects of certain nutrients on the susceptibility of nasturtium to visible injury by HCl

Methods

This was a group of experiments conducted by Mr. Terry R. Thomas and reported in a master's degree thesis (Thomas, 1976) entitled: "The Effects of Calcium, Magnesium, Potassium, and Chlorine Nutrition on Susceptibility to Visible Injury of Nasturtium by Hydrogen Chloride Gas."

The nasturtium plants were grown hydroponically with the nutrients under study circulating around the plant roots. Plants were exposed

to HCl for 20 minutes and injury consisted of irregular interveinal necrosis (N), frequently with glazing (G) on the abaxial surface, and leaf discoloration (D) on the adaxial surface. This injury varied, but a useful damage index was DI = 2 N% + G% + D%, where % was the percent leaf area injured. Also considered were the effects of dark and/or dew formation as conditions prior to or during fumigations.

Results

The nasturtium plants were 34 days old and usually had seven leaf sets when exposed to HCl gas. Injury occurred at 18-26 mg/m^3 HCl, so this concentration range was used for all experiments. The frequency and severity of injury on each leaf was noted. The third leaf set (seventh was youngest) had visible injury more frequently (70%) than other sets. This set was almost mature and nearly fully expanded when plant was fumigated. Youngest leaf sets exhibited injury least frequently (6%) while the injury frequency of the other leaves were normally distributed. The fourth set was most severely injured, as based on average necrosis per leaf. Again there was a normal distribution of the injury severity on the other leaves with the very youngest leaves having only trace necrosis.

The plant sensitivity to HCl damage was directly proportional to calcium in the nutrient solution. Calcium content of the dry weight also increased with damage. Plants deprived of adequate magnesium were more susceptible to HCl injury. Magnesium-deficient plants had some injury even without exposure to HCl. Potassium did not seem to affect sensitivity. When total nutrition was reduced, there was a slight increase in glazing and discoloration, but not much effect on total injury. There was a direct linear relationship between damage and chlorine ion concentration in the nutrient solution.

Plants exposed to HCl in the dark following three to four hours pre-incubation in a dark dew chamber sustained more severe damage than if pre-incubation occurred in a dark dry chamber or in the light. The injury on plants with dew was a necrotic spotting which was more indiscriminate than the injury on dry plants exposed to HCl in the light. After a dark treatment and HCl fumigation, more chlorine was found in the dry weight of the dew treated plants than the dry treated plants.

Discussion

There is evidence here that the sensitivity of nasturtium plants to HCl gas is affected by the plant's nutritional milieu. Although it is difficult to relate these experiments to field conditions, it is probably true that otherwise healthy plants with adequate fertilization will probably better withstand exposures to HCl gas. There may be complications if the plants are overfertilized.

The dew treatments indicate the probability that pre-dawn exposures to HCl of plants near the coast would cause more severe damage than exposures occurring later in the day when the plants are dry.

B. Effect of nutrition level on the sensitivity of marigold and nasturtium plants to HCl gas

Methods

Test populations of marigold and nasturtium were divided into three groups each. During the growing period, the three groups received 40, 80, and 160 ml of a balanced nutrient solution (Hoagland and Arnon, 1938) per pot per week, respectively. The normal nutrient supplement for plants of this size is 80 ml per week. The marigolds and nasturtiums received nutrient treatment for ca. five and six weeks, respectively, before subgroups of the plants were exposed to HCl gas for 20 minutes. Twenty-four to 48 hours after exposure to the pollutant, the plants were checked for visible injury. A damage index related the leaf area damaged to total leaf area. The roots and tops of the treated plants were then separated and oven-dried for 72 hours at 70 C before weighing. Dry weights were compared to dosage and nutrient treatments.

Results

After five to six weeks of the nutrient treatments, the plants appeared uniform. Table 3 summarizes the parameters and compares the responses. Interestingly, the marigold data indicated decreased growth with increased nutrient concentration, although only the buds/plant data were statistically significant in this regard. On the other hand, the nasturtium plants show strong positive reaction to the increased nutrient treatments in all three categories checked.

Tables 4 and 5 detail the measurements taken after the plants were exposed to HCl gas at the three concentrations listed. Visual damage has been indexed in the tables and Figures 7 and 8. After separating roots from plant tops, and oven-drying these parts, the dry weight was recorded and a summary is in the tables and in Figures 9 through 14. The marigold and nasturtium data were handled separately in analysis of variance manipulations with results presented in Table 6. As can be seen, the damage index is significant in both plant species and both the fumigation and nutrient treatments are of importance. There is significance among the dry weight data, but this is seen more often in the nutrient than in fumigation treatment, and nasturtiums were apparently more sensitive than marigolds to the double stress of both treatments.

Since the dry weights were measured within two or three days of exposure, significant differences at the fumigation levels were surprising. The differences found due to the nutrient treatment were expected since it was the culmination of weeks of growth with the treatment.

Marigold plants showed less injury as nutrient level increased (40 to 160 ml), suggesting that tolerance was associated with nutrient treatment. When the plant dry weights were considered, there was a general decrease in plant biomass with increased concentration of fumigant. The exposed plants in the 160 ml nutrient treatment were larger than

Table 3. Response of marigold and nasturtium plants to treatments of balance nutrient solutions

Plant Response	Nutrient Treatment		
	40 ml	80 ml	160 ml
	Marigold Plants[2]		
Height (cm)	19.1 A[3]	19.0 A	18.9 A
Number of leaves	8.9 A	8.8 A	8.6 A
Number of buds/plant	0.3 A	0.2 B	0.2 B
	Nasturtium Plants[4]		
Total number of leaves	27.2 a[5]	35.4 b	38.5 c
Number of main leaves	12.0 a	13.4 b	13.7 b
Number of axillary leaves	15.2 a	22.1 b	25.1 c

[1]Nutrient treatment consisted of a weekly application of 40, 80, or 160 ml of Hoagland's solution per pot.

[2]Senator Dirkson Marigold plants were all 45 days old.

[3]Each value is the mean of 80 plants. Means in each response category row followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[4]Dwarf double primrose yellow nasturtium plants were all 36 days old.

[5]Each value is the mean of 60 plants. Means in each response category row followed by the same letter are not significantly different at the 1% level according to Duncan's multiple range test.

Table 4. Response of marigolds to three treatments of nutrient solution and three doses of HCl gas

Response Measurement	HCl dose[1]	Nutrient Treatment[2]		
		40 ml	80 ml	160 ml
Damage Index	Control	0.0[3]	0.0	0.0
	Low	0.03	0.02	0.01
	Medium	0.11	0.06	0.03
	High	0.20	0.17	0.11
Root dry weights(g)	Control	0.38[4]	0.46	0.24
	Low	0.51	0.50	0.52
	Medium	0.32	0.35	0.35
	High	0.31	0.36	0.36
Shoot dry weights(g)	Control	1.21[4]	1.32	1.40
	Low	1.08	1.28	1.49
	Medium	1.04	1.21	1.47
	High	0.99	1.16	1.34
Plant dry weights(g)	Control	1.59[4]	1.78	1.64
	Low	1.59	1.78	2.01
	Medium	1.36	1.56	1.82
	High	1.30	1.52	1.70

[1]HCl doses represented as Low, Medium, and High are 20-minute exposures at 12, 22, and 31 mg/m^3.

[2]Nutrient treatment is ml Hoagland's solution per pot per week.

[3]Damage index is the ratio of leaf area exhibiting damage symptoms to total leaf area. Each value is the mean of 25 plants.

[4]Each value is the mean dry weight in grams of 25 plants.

Table 5. Response of nasturtium to three treatments of nutrient solution and three doses of HCl gas

Response Measurement	HCl dose[1]	Nutrient Treatment[2]		
		40 ml	80 ml	160 ml
Damage Index	Control	0.0[3]	0.0	0.0
	Low	0.0	0.0	0.0
	Medium	0.01	0.0	0.0
	High	0.13	0.03	0.03
Root dry weights(g)	Control	0.24[4]	0.41	0.39
	Low	0.24	0.30	0.36
	Medium	0.30	0.47	0.40
	High	0.35	0.52	0.45
Shoot dry weights(g)	Control	0.79[4]	1.45	1.51
	Low	0.64	0.77	1.06
	Medium	0.80	1.12	1.21
	High	0.68	0.99	1.06
Plant dry weights(g)	Control	1.03[4]	1.86	1.89
	Low	0.88	1.07	1.42
	Medium	1.10	1.58	1.61
	High	1.03	1.51	1.66

[1]HCl doses represented as Low, Medium, and High are 20-minute exposures at 4, 15, and 26 mg/m^3, respectively.

[2]Nutrient treatment is ml Hoagland's solution per pot per week.

[3]Damage index is the ratio of leaf area exhibiting damage symptoms to total leaf area. Each value is the mean of 15 plants.

[4]Each value is the mean dry weight in grams of 15 plants.

Table 6. Analysis of variance for nutrient and fumigation studies with marigolds and nasturtiums

Variable	Matrix	Significance[1]	
		Marigold	Nasturtium
Damage index[2]	Fumigant level[3]	**	**
	Nutrient level[4]	**	**
	Fumigant x nutrient level	**	**
Root dry weight(g)	Fumigant level	**	**
	Nutrient level	NS	**
	Fumigant x nutrient level	**	NS
Shoot dry weight(g)	Fumigant level	NS	**
	Nutrient level	**	**
	Fumigant x nutrient level	NS	**
Plant dry weight(g)	Fumigant level	**	**
	Nutrient level	**	**
	Fumigant x nutrient level	NS	**

[1]** denotes significance at 1% level, * denotes significance at 5% level, NS denotes no significance.

[2]Damage index is the ratio of leaf area exhibiting damage symptoms to total leaf area. Where there was more than one axillary leaf at a particular node (nasturtium), the damage index of the most severely injured leaf was taken to represent all injured axillary leaves at that node.

[3]Fumigant treatment was HCl gas supplied for 20 minutes at one of three concentrations: marigolds, 12, 22, or 31 mg/m^3; nasturtiums, 4, 15, or 26 mg/m^3.

[4]Nutrient treatment consisted of weekly applications of 40, 80, or 160 ml of Hoagland's solution per pot.

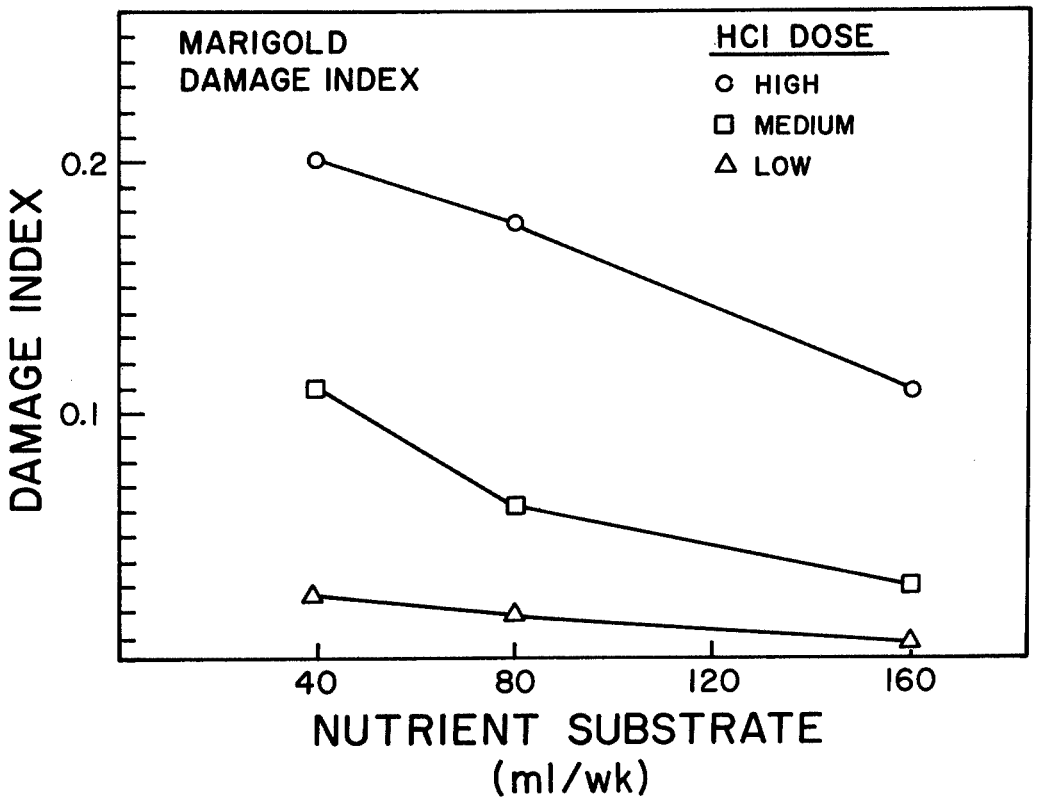


Figure 7. HCl-induced damage on marigolds grown with nutrient supplements before exposure.

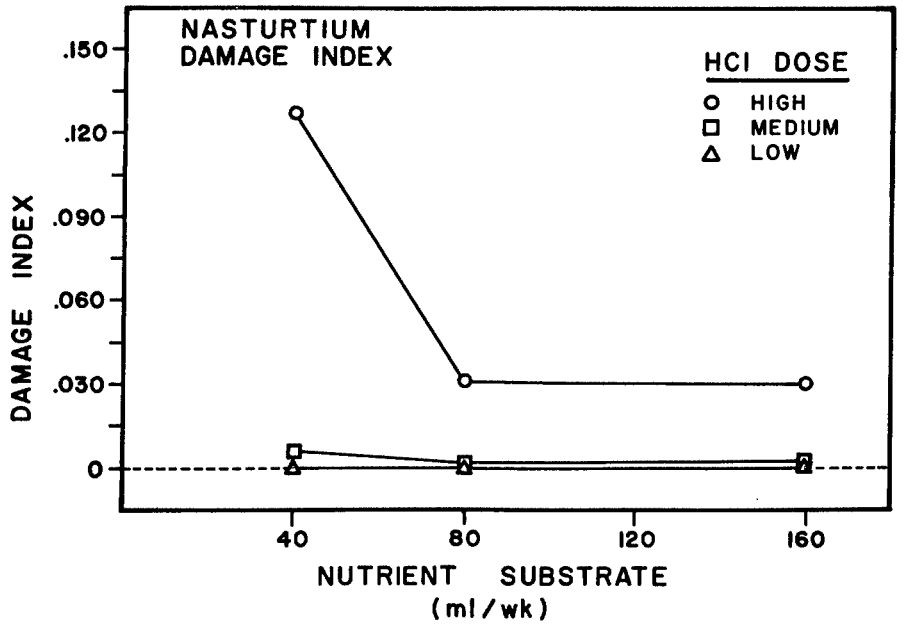


Figure 8. HCl-induced damage on nasturtium plants grown with nutrient supplement before exposure.

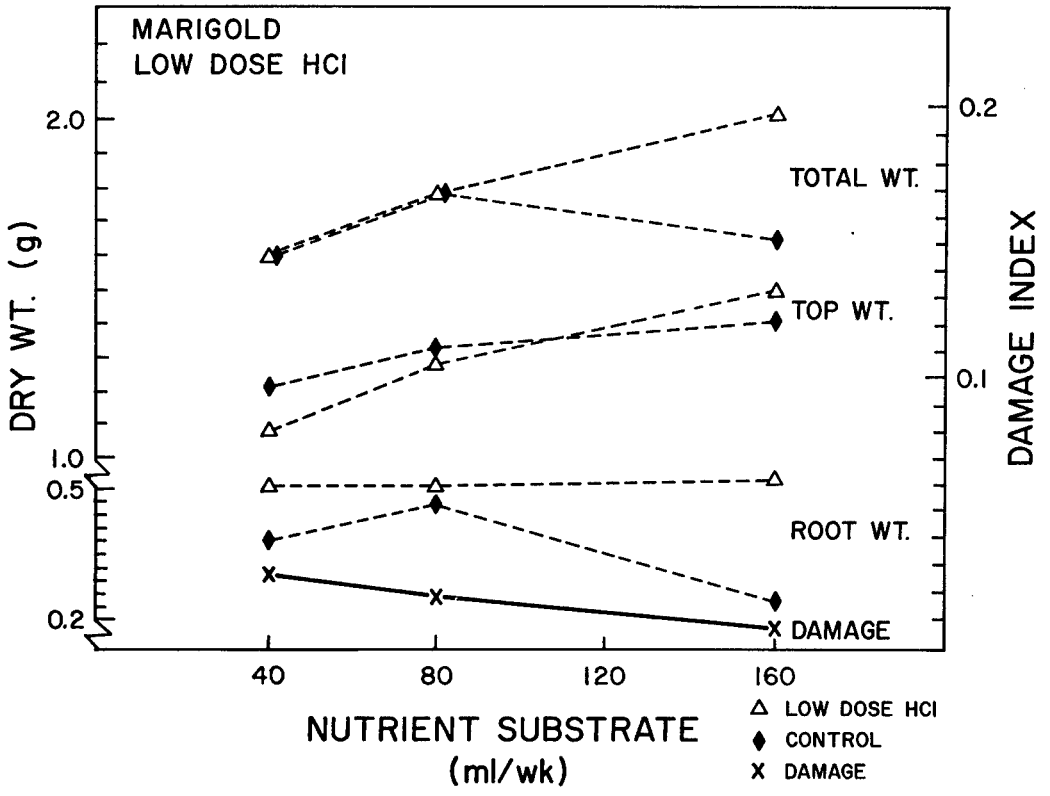


Figure 9. Damage and dry weights of marigold plants grown with nutrient supplements and then exposed to 12 mg/m^3 HCl for 20 minutes.

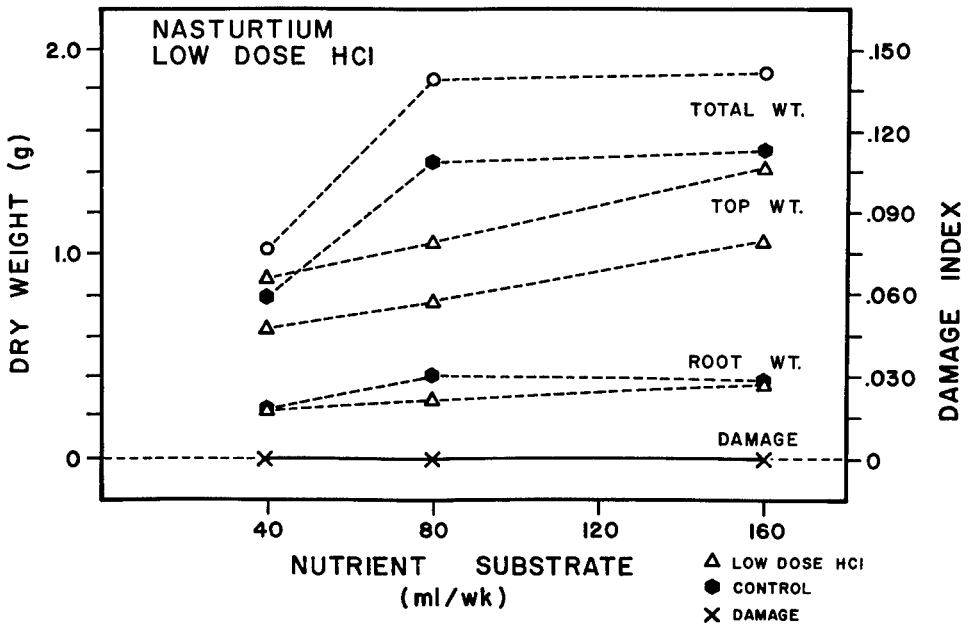


Figure 10. Damage and dry weights of nasturtium plants grown with nutrient supplements and then exposed to 4 mg/m^3 HCl for 20 minutes.

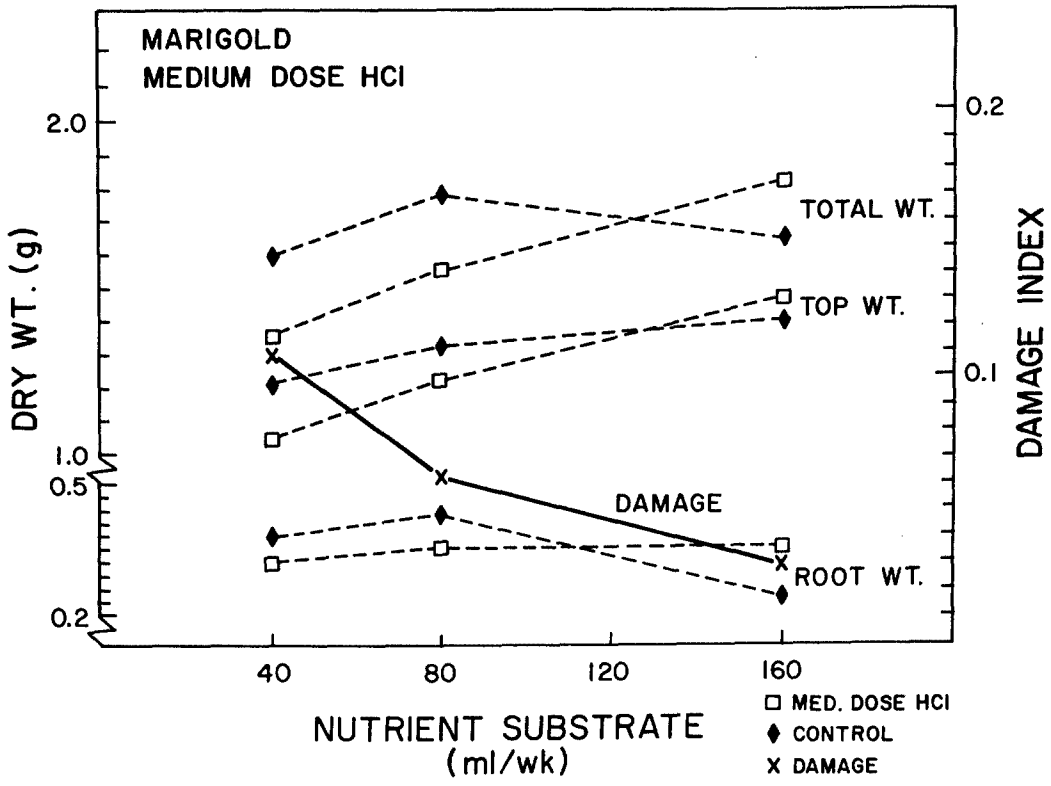


Figure 11. Damage and dry weights of marigold plants grown with nutrient supplements and then exposed to 22 mg/m^3 HCl for 20 minutes.

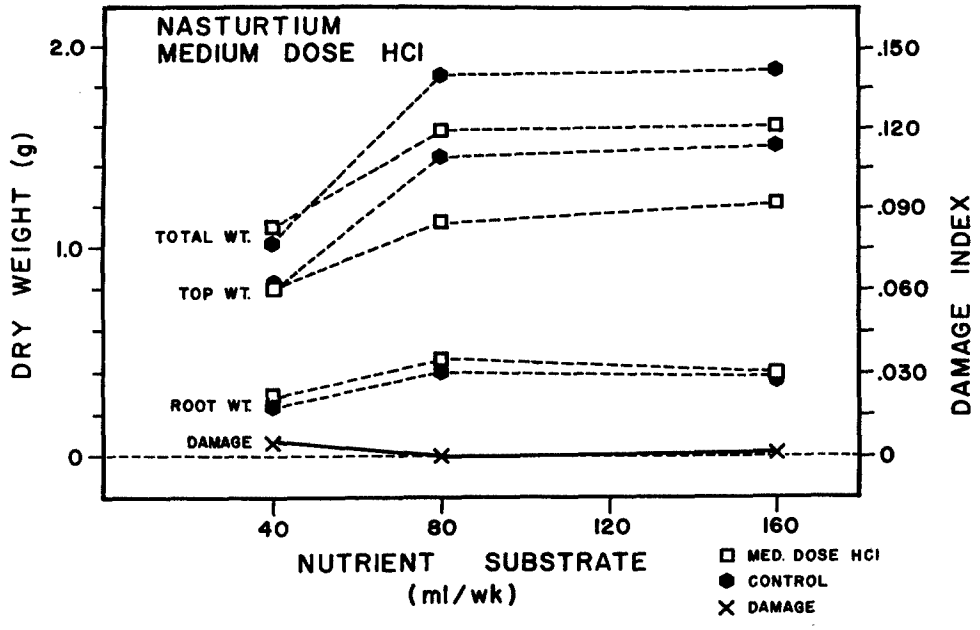


Figure 12. Damage and dry weights of nasturtium plants grown with nutrient supplements and then exposed to 15 mg/m^3 HCl for 20 minutes.

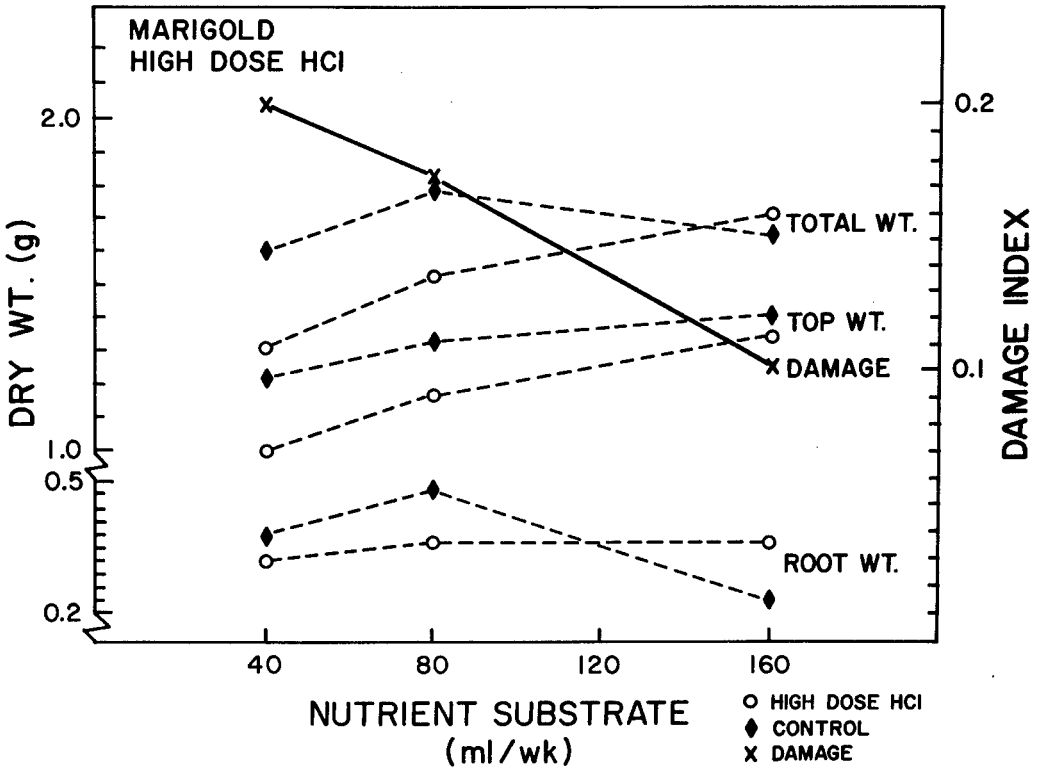


Figure 13. Damage and dry weights of marigold plants grown with nutrient supplements and then exposed to 31 mg/m^3 HCl for 20 minutes.

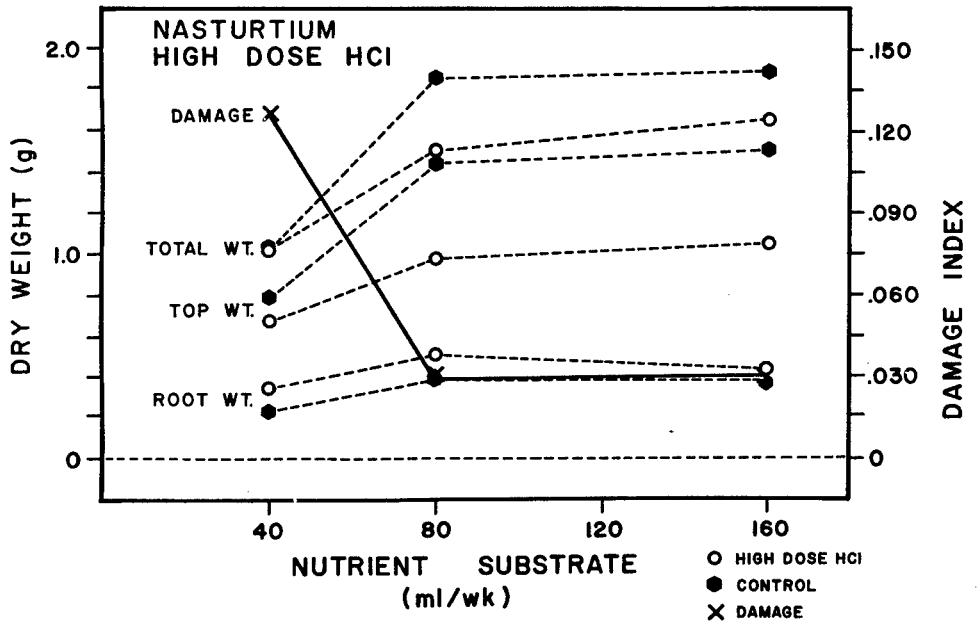


Figure 14. Damage and dry weights of nasturtium plants grown with nutrient supplements and then exposed to 26 mg/m^3 HCl for 20 minutes.

unexposed controls. Apparently the HCl stimulates plant growth under these conditions. If shoot/root ratios are considered, both fumigant and nutrient effects seem to be on the shoots.

Nasturtium plants were generally more tolerant than marigolds to visible injury at the levels of HCl used. The high level HCl, however, caused less damage on plants treated at the 80 and 160 ml nutrient levels. Nasturtium plants treated with HCl all had a smaller biomass than the controls. As HCl exposure concentrations increased, however, the biomass of the 160 ml nutrient-treated plants did also. There does not appear to be a dose related response with nasturtium, perhaps due to lower HCl concentrations during exposures compared to the concentration to which the marigolds were exposed. Considering shoot/root ratios, increasing nutrients affects the shoot while the increasing pollutant concentration affects the roots.

Discussion

This experiment demonstrated that the overall nutrition supplied to some species can affect the tolerance of the plant to injury induced by short exposures to HCl gas. The importance of careful control of nutrition during growth of experimental plants is thus stressed. This was conducted under greenhouse conditions prevalent in the winter and early spring months and the lower light levels and cooler temperatures may have affected plant growth. It would be useful to know whether the same relationships of nutrition and pollution tolerance would be operative under field conditions, or under more severe temperature conditions.

C. Tomato and barley development, productivity, and exposure studies

Methods

A large population of tomato and barley plants were grown from seed. Two varieties of tomato, Ace, a true line, and 6718, a hybrid, were selected and fumigated at the seedling, vegetative, and reproductive stages. The barley plants were exposed to toxicants at emergence, tillering, stem elongation, and heading. All plants were exposed to 20 minutes of HCl, HF, and HCl plus HF at phytotoxic levels. Plant height, number of leaves, and number of fruit spurs were recorded weekly for the tomato plants. At harvest, 78 days after seeding, height, total number of blossoms, and number and weight of immature fruit were recorded. Dry weights of the plant leaves, tops, and roots were also obtained. Barley plants were measured weekly and height, number of leaves, and number of tillers were recorded. After the plants matured, the number of heads and seeds were counted as a measure of yield. Tables 7 and 8 detail the variables in the experiment.

Table 7. Variables in tomato productivity experiment

Tomato Varieties	Growth State When Fumigated	Fumigant	Weekly Data	Final Data
Ace, true line	Seedling, 20-day-old	HCl	Height	Height
6718 VF, hybrid	Vegetative, 40-day-old	HF	No. of immature fruit	No. of leaves
	Flowering, 60-day-old	HCl + HF	No. of flowers	No. of flowers
		No treatment control	No. of inflorescences	No. of fruit
				Stem dry weight
				Leaf dry weight
				Roots dry weight

Table 8. Variables in barley productivity experiment

Barley Variety	Growth Stage When Fumigated	Fumigant	Weekly Data	Final Data
CM 69	Emergence, 1-week-old	HCl	Height	No. of heads
	Tillering, 6-week old	HF	No. of leaves	Top dry weight
		HCl + HF	No. of tillers	Root dry weight
		No treatment control	Injury rating	Head dry weight
			No. of heads	Seed dry weight

Results

Considering the tomato data first, Figure 15 illustrates the mean values for the plant heights. Of particular interest is that the heights begin to diverge after the fumigations are made. Although one or another of the fumigated groups were usually taller than the controls, the differences are not significant. The hybrid variety generally grew taller than the true seed line.

Tables 9A and 9B summarize the treatments, stage of growth at fumigation, fumigant concentration, and growth data at harvest. Selected growth data have been statistically compared among the stage treatments and the same letter following two numbers within the group shows that they are not significantly different at the 5% level by Duncan's multiple range test. The growth data are also charted in Figures 16 through 19. The arrow marks age when plant was exposed to pollutants. Final height of the tomato plants showed that the Ace controls were usually shorter than the fumigated plants, and that the hybrid control plants were usually taller.

From Tables 9A and 9B and Figure 16, it can be seen that there were no significant differences among number of leaves with the Ace tomato plants and only small differences among the hybrid plants. The plants had significantly more leaves after exposure to HF or HCl + HF during vegetative and flowering stages.

No trends were evident in the fruit and flower data, Figure 17, unless the ratio of fresh weight per fruit was considered. Then it appeared that the early fumigation reduced the fruit yield. The HCl and HF fumigation was particularly severe on the hybrid fumigated at the vegetative stage. Exposures during flowering were much less severe on the true line than on the hybrid plants. No flowers were present during the earlier fumigations while later they were present and might have been damaged. The data in Figure 18 and part of Tables 9A and 9B suggest that the fresh weight of the immature fruit was more affected by the earlier exposures during seedling or vegetative stages when the fruit was not present. Less differences were noted when plants were exposed during flowering. The hybrid variety seemed more productive, but these late fumigations tended to cancel any superiority in yield.

Figure 18 is a combination of much of the dry weight data presented in Tables 9A and 9B. There seemed to be little difference in dry weights and the roots were similar in weight. The ratios of the shoot to root dry weights and root to total dry weights are summarized in Table 10. There was no significance between growth stage groups of the Ace tomato plants and the only differences among the hybrid line were confined to the first two fumigations.

In the case of barley, growth data were taken over a much longer period since heads were allowed to mature completely and the plant was essentially dead when harvested. Figures 20 through 23 detail the growth and decline of the plants, illustrating the height, number of leaves, and number of tillers, respectively, of the four groups of plants

Table 9A. Ace tomato responses to 20-minute exposures of HCl and HF at different growth rates

Treatments			Growth Data at Harvest[1]								
Age (Days)	Pollutant	Concentration (mg/m^3)	Height (cm)	No. of Leaves	No. of Flowers & Fruit	Fresh Weight/ Fruit (g)	Fresh Weight/ Flowers & Fruit (g)	Dry Weight Leaves (g)	Dry Weight Stems (g)	Dry Weight Roots (g)	Dry Weight Total (g)
--	Control	0	58	11.4 A[2]	3.7	48 A	13.0	8.2	3.9	2.1 A	14.2 A
20	HCl	53	76	11.3 A	4.1	10 B	2.4	9.4	3.8	2.0 A	15.2 A
20	HF	11	72	11.9 A	5.1	9 B	2.2	8.9	3.6	2.1 A	14.6 A
20	HCl+HF	59+12	70	11.8 A	4.0	3 B	0.8	9.3	3.0	2.1 A	14.4 A
--	Control	0	58	11.4 A	3.7	48 A	13.0	8.2	3.9	2.1 A	14.2 B
41	HCl	45	70	12.1 A	4.6	16 B	3.4	10.5	4.6	2.2 A	17.3 A
41	HF	9	65	12.3 A	5.8	15 B	2.6	9.6	3.5	1.9 A	15.0 B
41	HCl+HF	46+11	57	11.3 A	4.8	11 B	2.3	8.6	3.1	1.9 A	13.6 B
--	Control	0	58	11.4 A	3.7	48 B	13.0	8.2	3.9	2.1 A	14.2 A
61	HCl	38	69	12.2 A	4.2	44 B	10.4	9.0	4.2	2.2 A	15.4 A
61	HF	8	70	11.3 A	3.0	69 A	23.0	7.5	3.2	1.9 A	12.6 B
61	HCl+HF	33+11	60	12.0 A	3.3	49 B	14.8	8.5	3.1	2.0 A	13.6 AB

[1]Plants were 77 days old at harvest.

[2]Means followed by the same letter are not significantly different from means in the same group (of 4) fumigated the same stage according to Duncan's multiple range test at the 5% level.

Table 9B. Hybrid 6718 VF tomato responses to 20-minute exposures of HCl and HF at different growth rates

Treatments			Growth Data at Harvest[1]								
Age (Days)	Pollutant	Concentration (mg/m^3)	Height (cm)	No. of Leaves	No. of Flowers & Fruit	Fresh Weight/ Fruit (g)	Fresh Weight/ Flowers & Fruit (g)	Dry Weight Leaves (g)	Dry Weight Stems (g)	Dry Weight Roots (g)	Dry Weight Total (g)
--	Control	0	77	11.6 A[2]	5.1	74 A	14.5	8.0	4.0	2.4 B	14.4 A
19	HCl	56	90	13.5 A	5.0	28 B	5.6	8.9	3.9	2.0 A	14.8 A
19	HF	14	86	12.6 A	6.6	23 BC	3.5	8.5	3.8	2.4 B	14.7 A
19	HCl+HF	60+13	88	12.1 A	7.2	13 C	1.8	8.3	3.3	1.9 C	13.5 A
--	Control	0	77	11.6 B	5.1	74 A	14.5	8.0	4.0	2.4 A	14.4 A
40	HCl	35	67	11.1 B	4.2	61 A	14.5	7.7	3.1	2.1 AB	12.9 A
40	HF	6	82	13.1 A	5.0	57 A	11.4	7.6	2.8	1.7 C	12.1 A
40	HCl+HF	45+7	74	12.4 AB	5.8	21 B	3.6	7.9	2.9	2.9 BC	12.8 A
--	Control	0	77	11.6 B	5.1	74 A	14.5	8.0	4.0	2.4 A	14.4 A
60	HCl	31	73	11.4 B	6.4	63 B	9.8	7.5	3.2	2.7 A	13.4 AB
60	HF	9	86	14.6 A	7.0	56 B	9.3	8.0	3.3	2.5 A	13.8 A
60	HCl+HF	41+9	80	14.3 A	4.1	29 C	7.1	7.3	2.8	2.0 A	12.1 B

[1]Plants were 77 days old at harvest.

[2]Means followed by the same letter are not significantly different from means in the same group (of 4) fumigated the same stage according to Duncan's multiple range test at the 5% level.

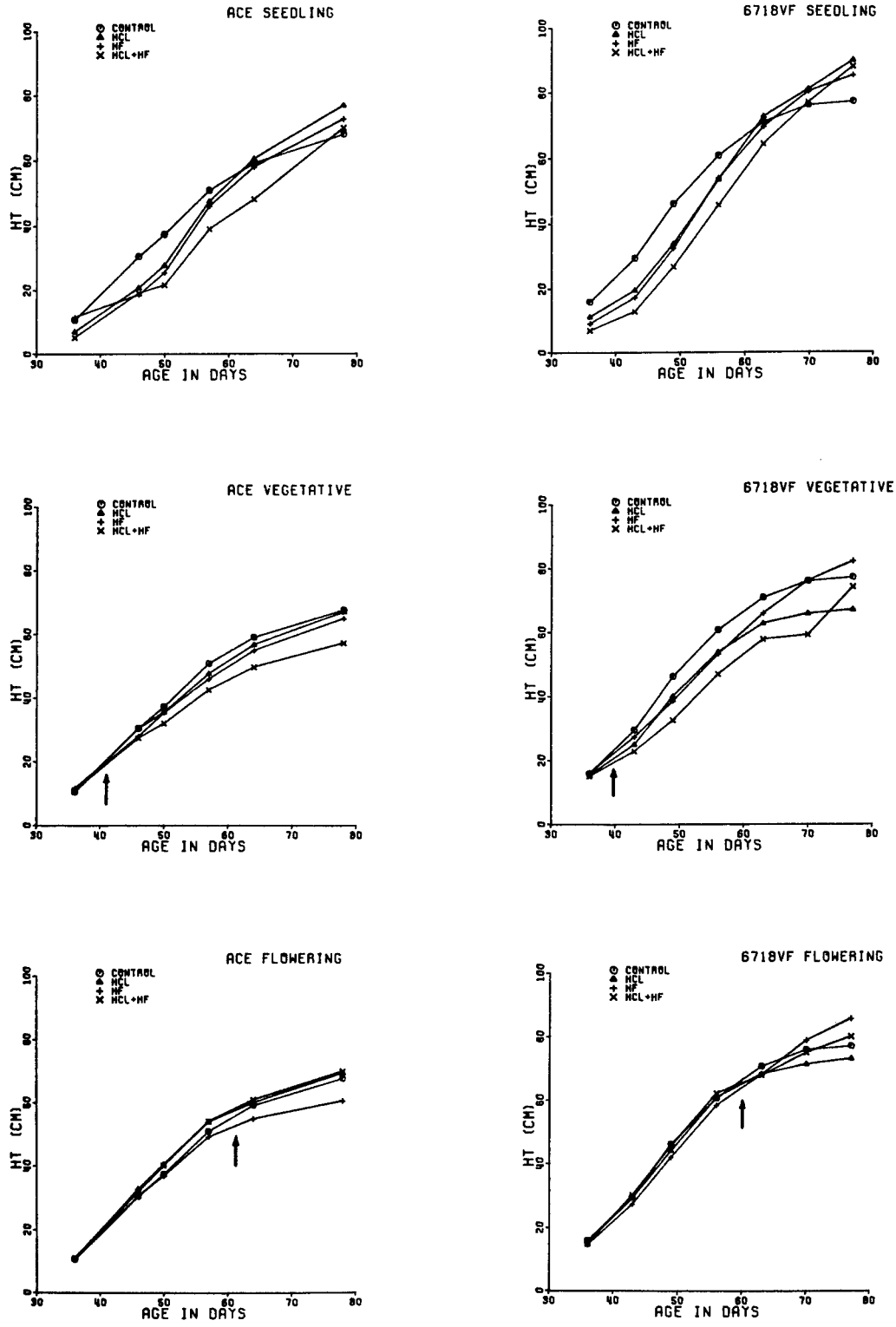


Figure 15. Weekly height measurements of Ace and 6718 VF tomato plants fumigated during seedling, vegetative, or flowering stages. Arrow indicates time of fumigation.

fumigated at each of the four stages of development. The arrow on each figure indicates the age when that group was fumigated. There was a decline in the height because the longer, older leaves died as the plant matured (see Figure 20). Fumigation during the stem elongation stage most affected height differences. The barley plants seem to mask or grow out of any height differences suffered during earlier fumigation periods and most of the leaves are dead or dying during the time of final fumigation.

When number of leaves were considered, the fumigations during tillering seemed most effective (see Figure 21); there was a randomness to the groups of the other curves. This is probably because the fumigations have little effect on the actual number of leaves, although parts of the leaves may be damaged by the toxicants.

Figure 22 considers the effect of fumigation on the numbers of tillers that the barley formed during development. The effects on tillering were similar to the leaf-number data. Although some effects may have been the result of fumigations during tillering or stem elongation, these may be merely a retardation of the growth of plants in one or another of the groups.

The last of the barley growth data graphs, Figure 23, illustrates how the fumigations effect the number of heads formed. It appears that heading was slightly delayed and that the final number of heads developed on plants exposed was reduced, especially in those plants exposed to HF during tillering or stem elongation. Fumigation during the heading stage did not seem to materially affect the numbers of heads formed.

Table 11 summarizes some of the data used for the previous figures. In addition, each category of growth data (for each fumigation) was tested by Duncan's multiple range test and the groupings are indicated by letters following the means. No height differences were found. No meaningful arrangements of the groupings of the other date were apparent. This table also summarizes the plant injury. Obviously, the HCl was not nearly as effective as the HF in damaging plants. The small amount of injury when plants were exposed to both HCl + HF during stem elongation as compared to the HF alone was unexpected. There was little growth data to record at the time of the fourth fumigation because the plants were starting to decline.

Table 12 summarizes the growth data accumulated when the barley plants were harvested. It was curious that a percentage of the heads formed were empty. If the seed weight per viable (or fully formed) head is calculated as in the last column, fumigated plants produced more than the controls.

Discussion

In the tomato studies, there seemed to be few differences between true line and hybrid, although the hybrid grew more vigorously. The vigor did not appear to bestow any tolerance. At the gas concentrations used

Table 10. Dry weight ratios for two varieties of tomato fumigated at different stages of development

Growth Stage	Shoot Dry Weight/Root Dry Weight				Root Dry Weight/Total Dry Weight			
	Treatment				Treatment			
	Control	HCl	HF	HCl+HF	Control	HCl	HF	HCl+HF
				Ace Tomato				
Seedling	6.23[1] A[2]	7.01 A	5.66 A	5.77 A	0.14 A	0.13 A	0.16 A	0.15 A
Vegetative	6.23 A	6.36 A	6.59 A	5.77 A	0.14 A	0.15 A	0.14 A	0.15 A
Flowering	6.23 A	6.14 A	5.39 A	5.93 A	0.14 A	0.14 A	0.16 A	0.15 A
				6718 Tomato				
Seedling	5.06 B	6.33 A	5.52 AB	6.07 A	0.17 A	0.14 B	0.16 AB	0.14 B
Vegetative	5.06 B	5.27 B	6.23 A	5.79 AB	0.17 A	0.16 AB	0.14 B	0.15 AB
Flowering	5.06 A	4.37 A	4.58 A	4.65 A	0.17 A	0.20 A	0.19 A	0.19 A

[1]Group means

[2]Means followed by the same letter are not significantly different from means in same group at same growth stage according to Duncan's multiple range test at the 5% level.

Table 11. Response of barley plants exposed to HCl and HF during one of three stages of plant development

Growth State	Plant Response	Treatment (mg/m^3)			
		Control (0)	HCl (50)	HF (50)	HCl/HF (30/50)
Emergence (fumigated 6 days after seeding)	Height (cm)	68.1A[1,2]	72.9A	72.7A	67.2A
	No. of leaves	26.6BCD	22.3CD	35.2ABCD	37.8AB
	No. of tillers	8.8BC	7.9C	11.4AB	11.7AB
	No. of heads	8.0BC	6.8CDE	7.6 BCD	8.4AB
	Injury rating[3]	0	6%	53%	49%
		Control (0)	HCl (30)	HF (30)	HCl/HF (30/30)
Tillering (fumigated 42 days after seeding)	Height (cm)	68.1A	71.1A	72.2A	68.7A
	No. of leaves	26.6BCD	30.6BCD	20.8D	30.5BCD
	No. of tillers	8.8BC	9.5BC	7.2C	9.3BC
	No. of heads	8.0BC	6.8CDE	5.5EF	6.3DEF
	Injury rating	0	Trace[4]	18%	19%
		Control (0)	HCl (30)	HF (22)	HCl/HF (30/22)
Stem elongation (fumigated 65 days after seeding)	Height (cm)	68.1A	73.8A	73.8A	68.2A
	No. of leaves	26.6BCD	46.3A	36.8ABC	35.0ABCD
	No. of tillers	8.8B	14.2A	9.8BC	9.9BC
	No. of heads	8.0BC	9.5A	5.3F	8.3AB
	Injury rating	0	5%	57%	14%

[1]Each value is the mean of 12 plants.

[2]Means in each variable category followed by the same letter are not significantly different at the 5% level, according to Duncan's multiple range test.

[3]The injury rating represents estimated area of plant tissue collapsed divided by total leaf area times 100. Damage evaluated 24 to 48 hours after gas exposure.

[4]The trace of HCl injury at tillering stage was faint chlorotic fleck or streaking on approximately 5% of the exposed leaf area.

Table 12. Barley response to 20-minute exposures of HCl and HF at different growth stages

Treatments			Growth Data at Harvest (22 weeks)						
			Dry Weight (grams)				No. of Heads		Seeds per Viable Head
Age[1] (wks)	Pollutant	Concen. (mg/m^3)	Tops	Roots	Heads	Seeds	Viable	Empty	
1	Control	0	10.1[2]	3.0	9.3	5.4	8.8[2]	2.6	0.61
1	HCl	50	10.0	2.0	10.3	6.4	8.0	2.3	0.80
1	HF	50	11.0	2.7	11.9	7.5	9.4	2.4	0.80
1	HCl+HF	30+50	10.7	1.9	10.2	6.4	8.5	3.7	0.75
6	Control	0	10.1	3.0	9.3	5.4	8.8	2.6	0.61
6	HCl	30	9.3	1.7	9.2	5.4	6.7	2.8	0.81
6	HF	30	8.0	1.4	6.9	4.7	5.7	0.8	0.82
6	HCl+HF	30+30	10.5	1.8	10.3	7.2	6.2	1.6	1.16
9	Control	0	10.1	3.0	9.3	5.4	8.8	2.6	0.61
9	HCl	30	11.2	1.9	9.9	5.6	8.4	5.5	0.67
9	HF	22	10.9	2.5	10.3	7.2	6.1	3.7	1.18
9	HCl+HF	30+22	9.2	1.9	10.2	6.9	8.8	2.1	0.78
17	Control	0	10.1	3.0	9.3	5.4	8.8	2.6	0.61
17	HCl	34	9.8	1.8	11.9	8.2	9.7	1.8	0.85
17	HF	26	10.5	1.7	10.4	6.5	10.4	2.0	0.62
17	HCl+HF	33+28	10.2	1.7	10.0	6.0	6.9	2.1	0.87

[1]Emergence, one week; tillering, six weeks; stem elongation, nine weeks; heading, 17 weeks

[2]Mean value of 10 plants

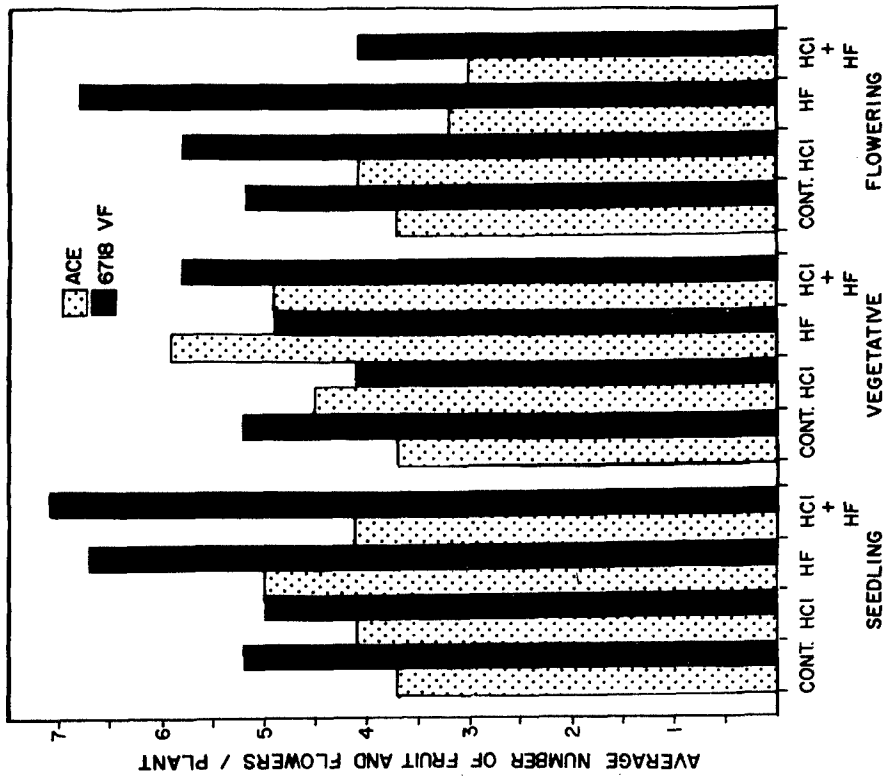


Figure 17. Average number of flowers plus fruit present on 75-day-old Ace or 6718 VF tomato plants fumigated once during growth.

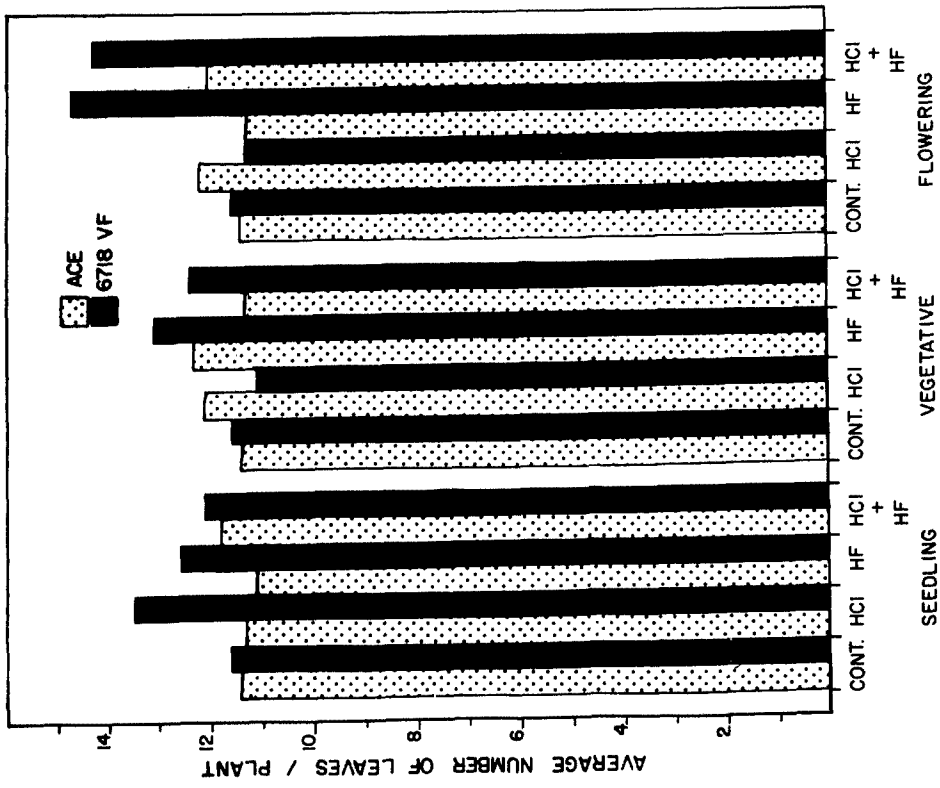


Figure 16. Average number of leaves on mature Ace or 6718 VF tomato plants previously fumigated during one of three growth stages.

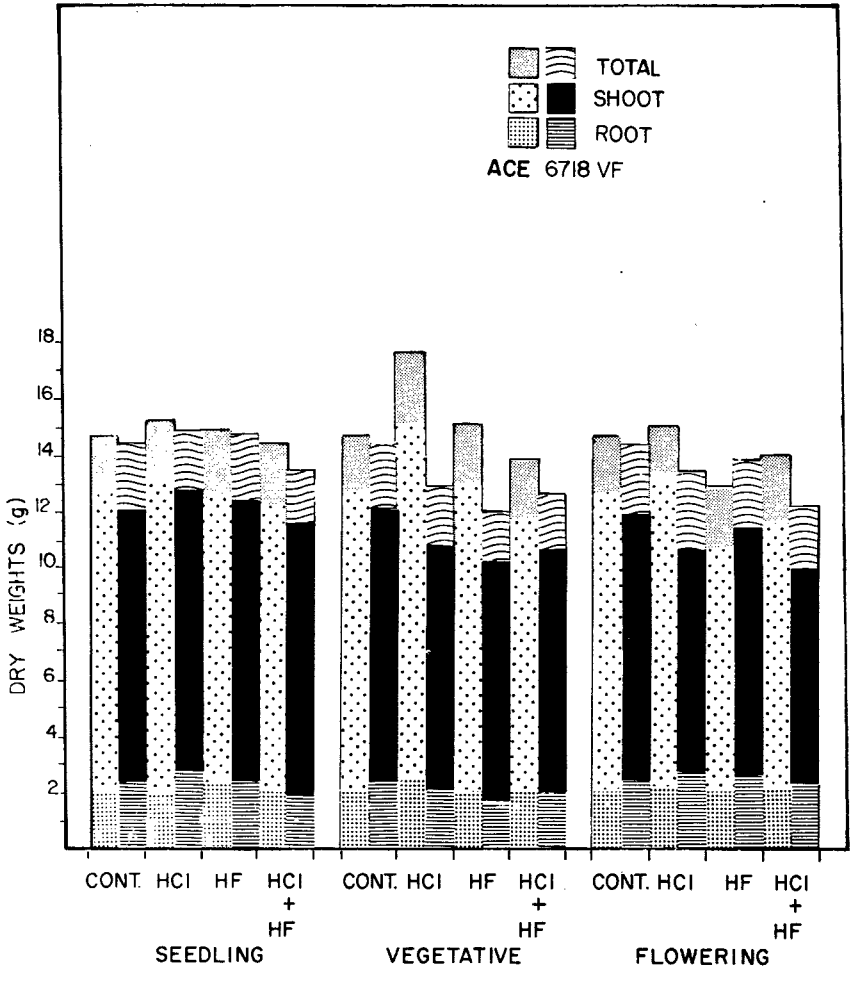


Figure 18. Dry weights of two tomato varieties exposed to pollutants during one of three growth stages and harvested 75 days after planting.

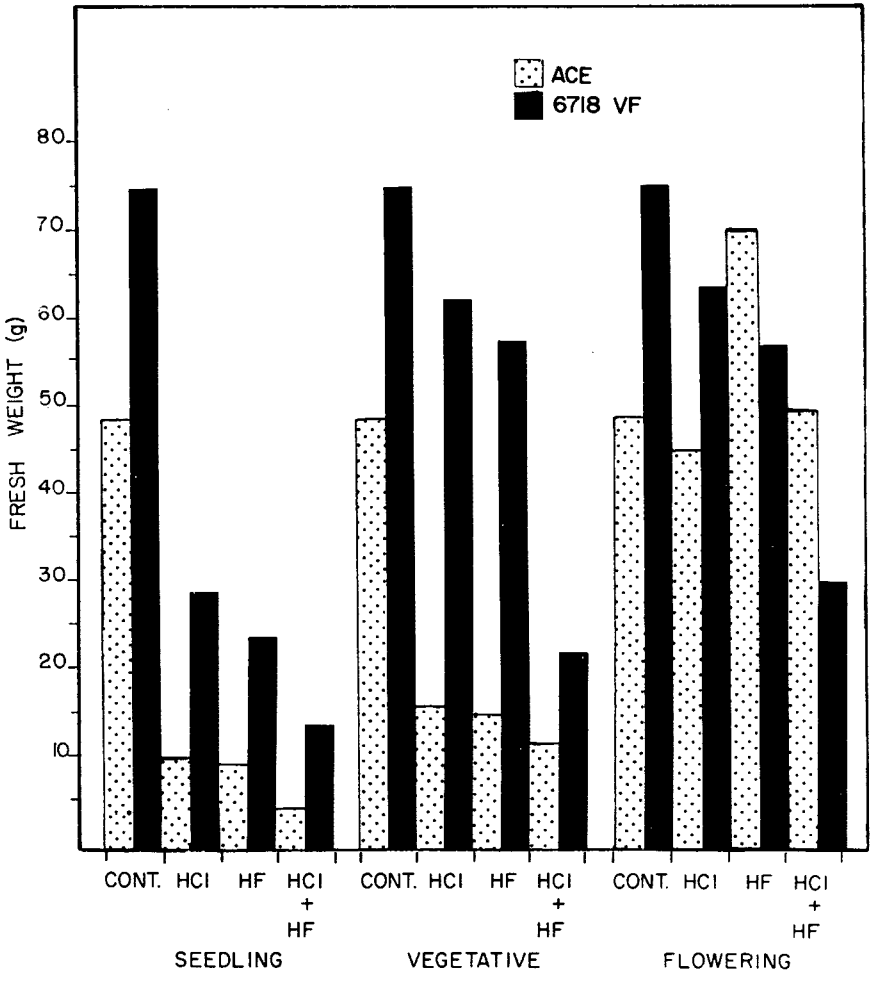


Figure 19. Fresh weight of immature fruit of 75-day-old tomato plants previously treated with pollutants once during growth.

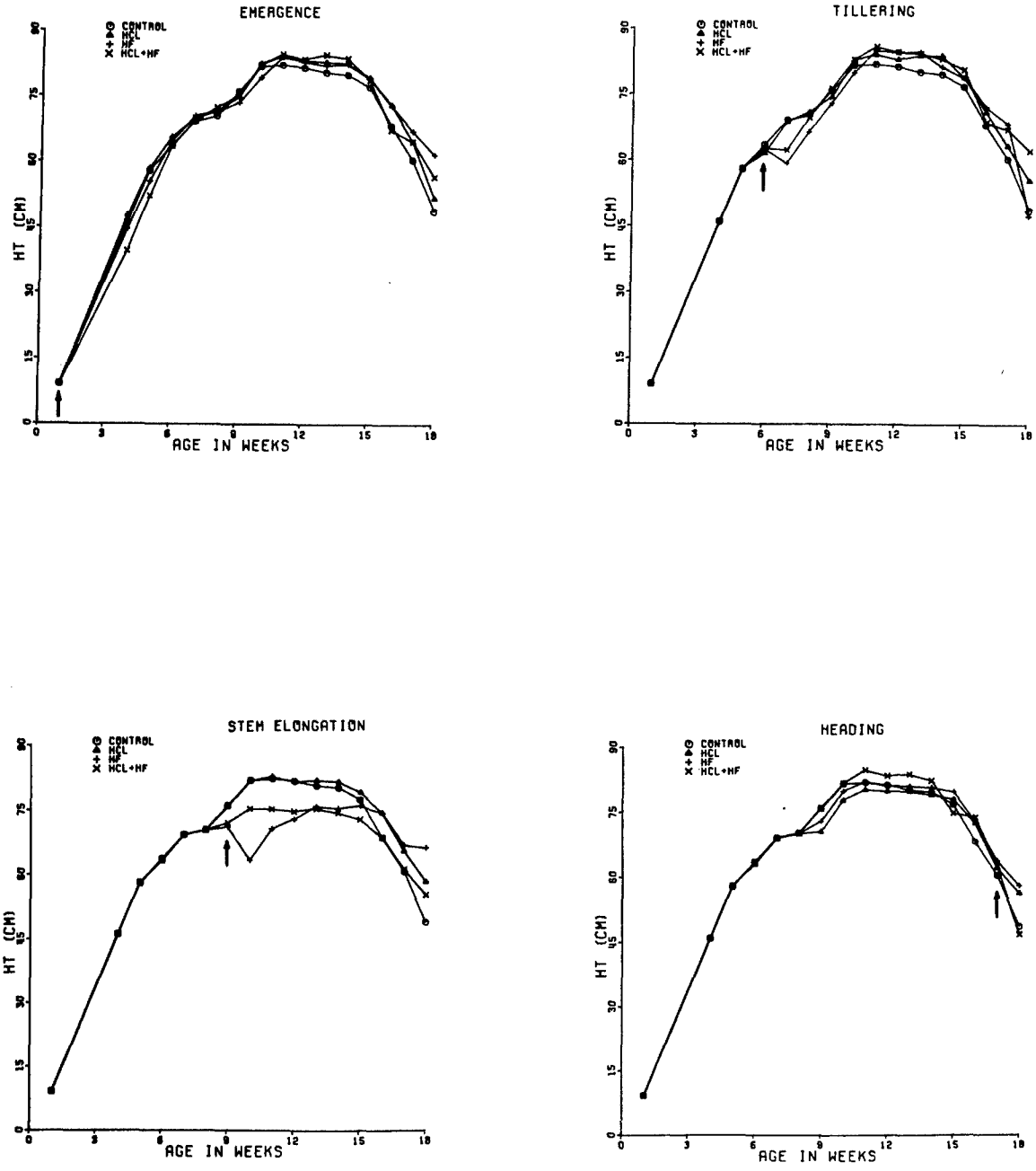


Figure 20. Weekly height measurements of barley plants fumigated during emergence, tillering, stem elongation, or heading. Arrow indicates time of fumigation.

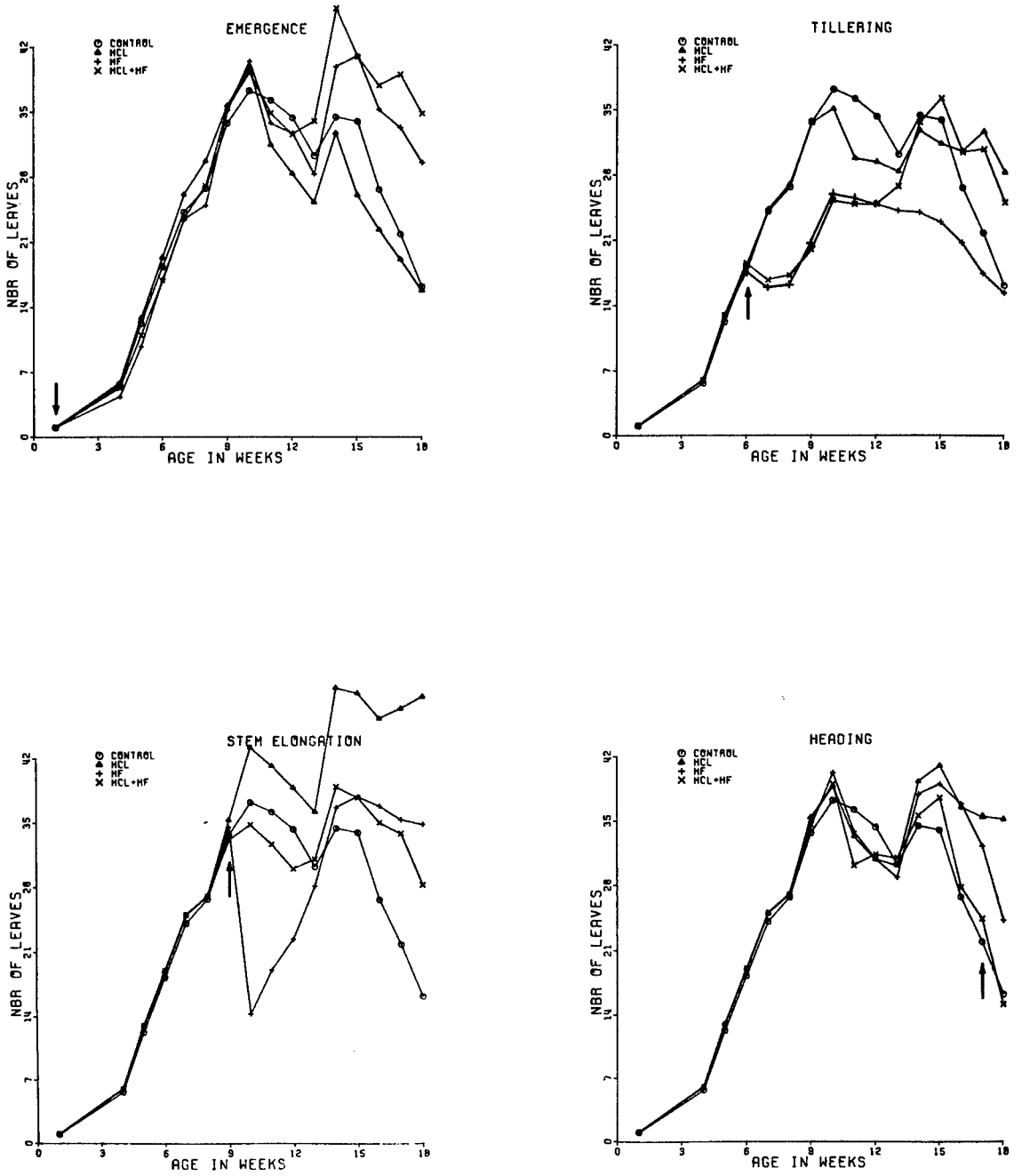


Figure 21. Average number of leaves on barley plants fumigated during one of four growth stages. Arrow indicates time of fumigation.

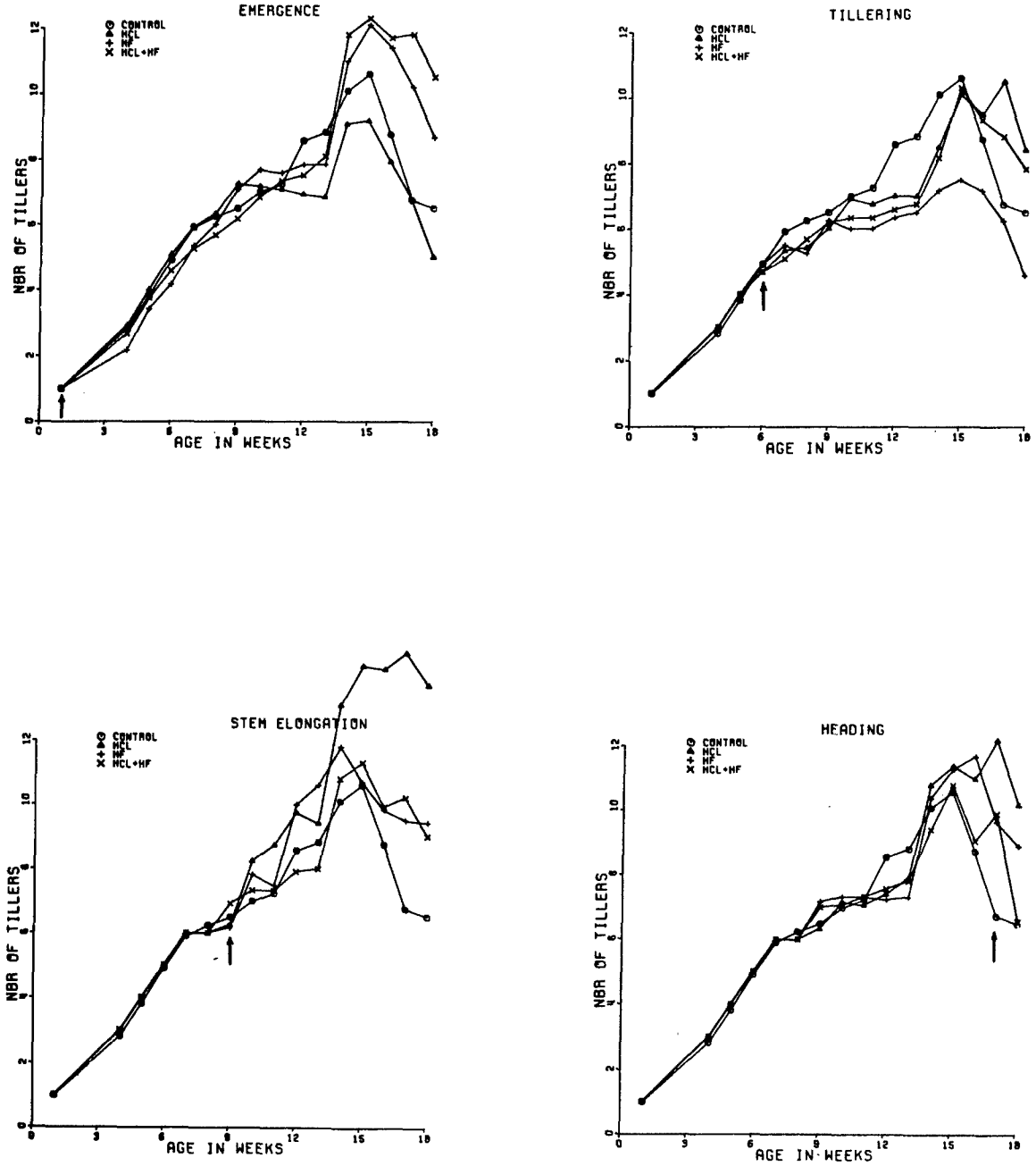


Figure 22. Average numbers of tillers on barley plants fumigated during one of four growth stages. Arrow indicates time of fumigation.

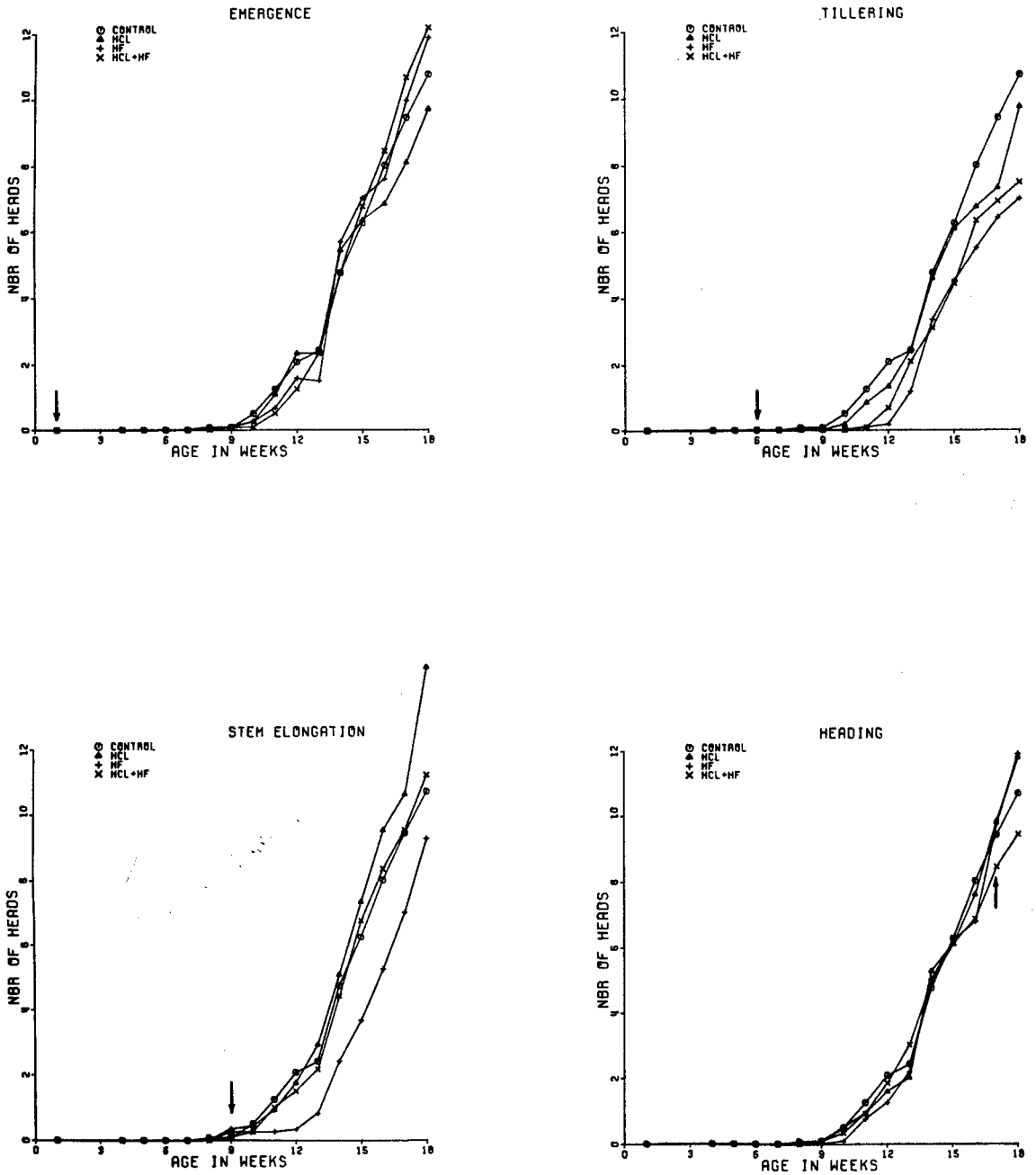


Figure 23. Average number of heads on barley plants fumigated during one of four growth stages. Arrow indicates time of fumigation.

in this study, no one stage of development was more sensitive. Roots did not appear affected by the exposures. The plants exposed at the younger ages appeared to have more serious yield losses. These losses, however, might only be a reflection of a retardation of plant growth. Had the plants been allowed to continue growing, later yields may have shown that all plants produce the same, within varieties.

In the barley experiments, plants at the young emergence stage were more sensitive to injury from the toxicants employed. The plants recovered from visible damage incurred during any of the growth stages. There seemed to be an increase in weight of seeds and the number of seeds per head with any of the toxicant combinations used compared to the nonfumigated controls.

D. Establishing threshold dosages for visible injury by HCl on different plant species

Methods

The six plant species under consideration were greenhouse grown to the two-to-six leaf state, about five weeks old. The plants were checked for uniformity and were grouped in lots of 12 of the same age and species. These were put into the racks to facilitate handling and to assure adequate spacing within the chamber. Placement in the rack was noted to detect any spot in the chambers where gas concentration might be higher; none were discovered. Exposures were made in the mid-morning to mid-afternoon to take advantage of fairly constant high light intensities. Very short exposures were conducted by sliding the rack of plants into a chamber without stopping the HCl generator. Most threshold work was limited to exposure times of five, 10, and 20 minutes. A few exposures at 15 and 30 minutes were conducted to verify predicted damage levels. After exposure the plants were removed from the chamber and returned to the greenhouse bench where they were observed after 24 to 48 hours.

Glazing and discoloration were visible at 12 hours and by 24 hours there was death of the severely damaged leaf tissue. Necrosis was visible 24 to 48 hours after exposure when the plants were graded and discarded.

Notes were made on the number of plants in the fumigation with any injury at all, the total number leaves with any damage, and a rough estimate of the percentage of leaf area damaged. Determinations of threshold were made by plotting injury or no injury per fumigation, but after a range was found, the leaf injury data were analyzed by computer. The computer program constructed "threshold curves" for one or more injured leaves per plant. A hyperbolic curve was constructed with high concentrations necessary for very short exposure times.

Results

The data accumulated thus far are presented in Figures 24 and 25. The circles (0) represent those fumigations of 12 plants in which no plants were damaged, whereas the crosses (+) represent fumigations where at least one of the plants was damaged. Table 13 summarizes threshold data to date.

Only enough data were available to produce the computer threshold curves for barley and bean exposures, Figure 25. Table 14 refers to the data used and the equation derived to plot the hyperbola of best fit.

Radishes proved to be a sensitive plant. Table 15 presents preliminary results on what concentrations and exposure times were tested. Although 264 plants were fumigated, the exposures were not definitive enough to produce limits for threshold damage.

Table 13. Threshold concentrations of HCl gas at which damage will occur on selected plant species exposed for short durations

Species	Exposure Time (minutes) 5	10	15	20	30
Barley	28[1]--40	16--28	13--24	10--23	9--21
Bean	21--36	14--26	12--26	11--25	
Centaurea	25	25	<30	<23	
Nasturtium	>50	30	<48	30	
Radish	22	16	12	10	
Tomato	>23	>22	>19	<15	

[1]Barley and bean data indicate the concentration of HCl in mg/m^3 necessary to produce zero-leaf and one-leaf injury as extrapolated from the computer-derived curves.

[2]Other species are estimates of threshold concentrations at mg/m^3. Symbols (>,<) refer to insufficient data above or below the level listed.

Table 14. Information for computer-derived threshold curve for barley and bean plants exposed to HCl

	Barley	Bean
Equation[1]	$I = -0.415 + 0.085(C) - \frac{9.588}{T}$	$I = -0.487 + 0.070(C) - \frac{5.003}{T}$
No. plants fumigated	672	504
Correlation coefficient (r)	0.517	0.662

[1]Where I = injury, C = concentration HCl in mg/m^3, and T = exposure time in minutes.

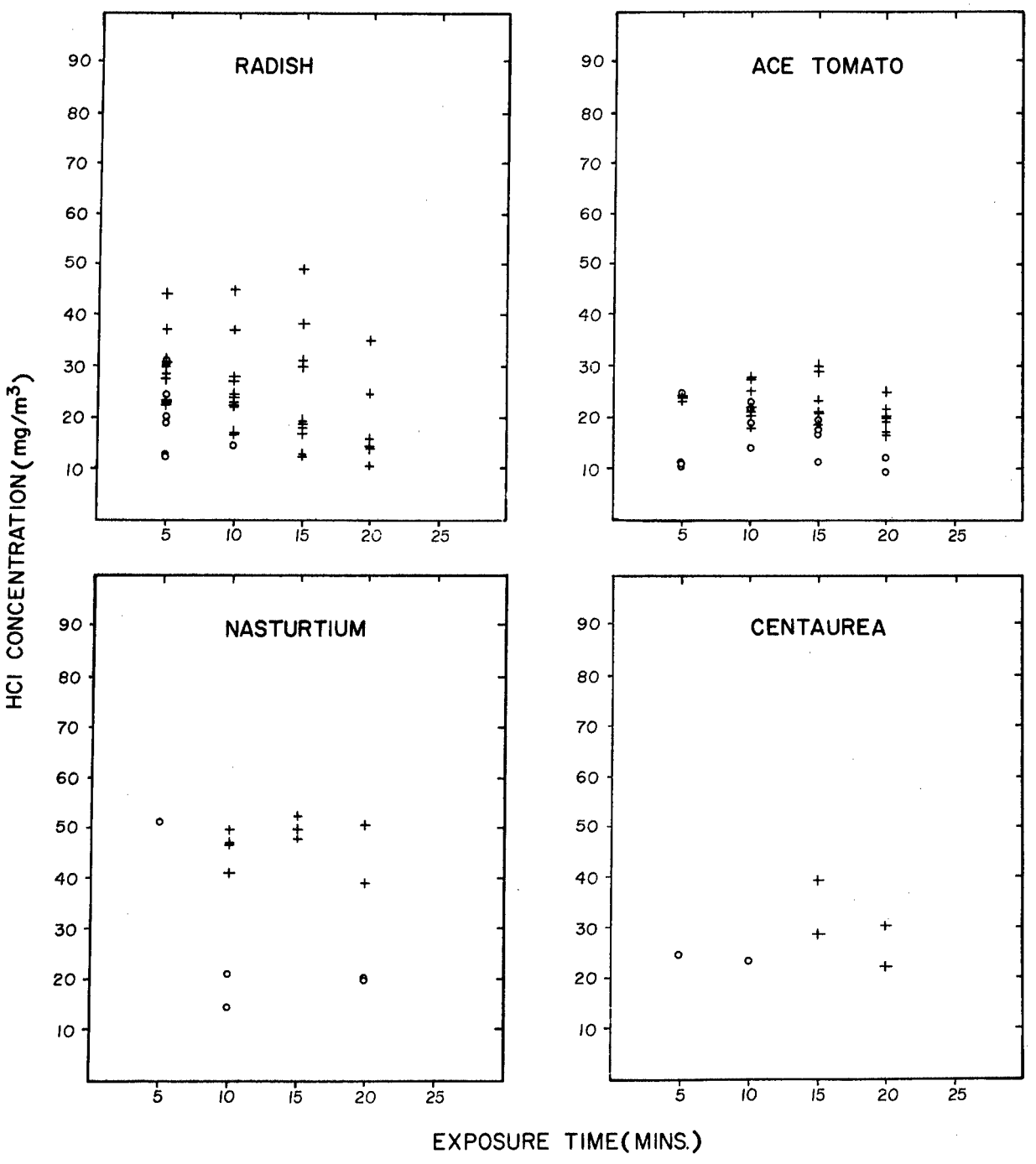


Figure 24. Response of radish, tomato, nasturtium, and centaurea plants to HCl gas. + = damage on 1 to 12 plants, o = no damage.

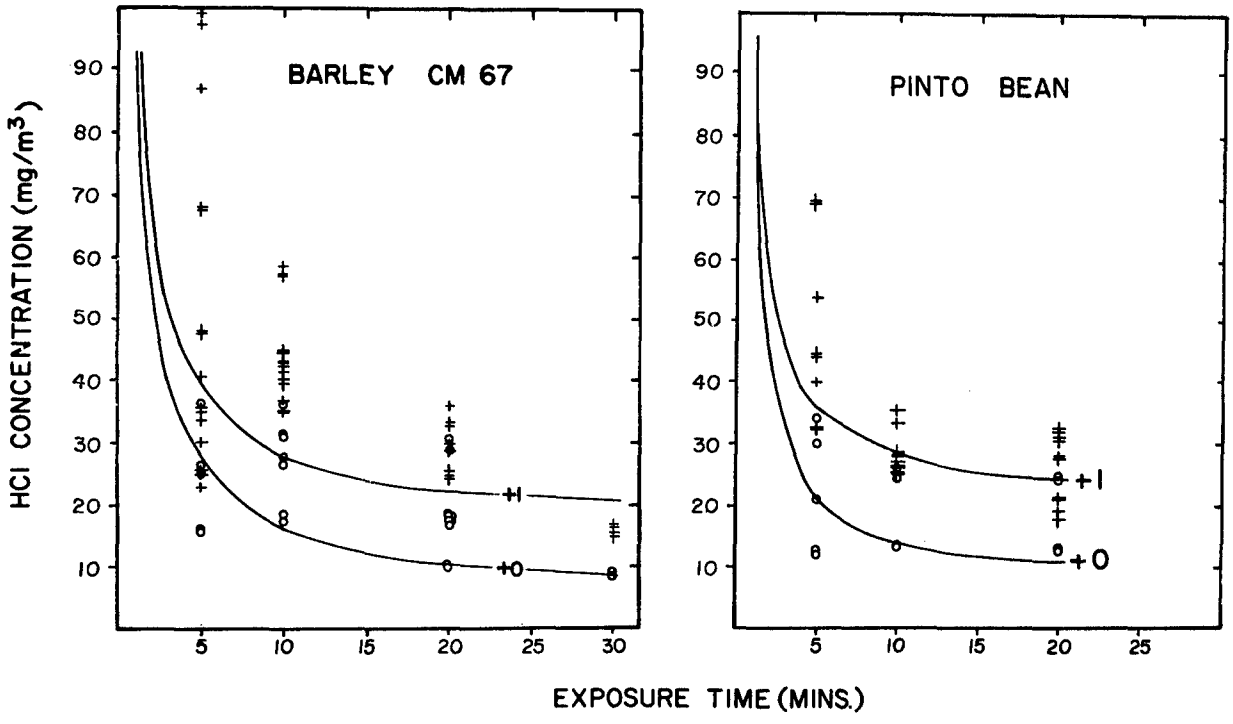


Figure 25. Response of barley and bean plants to short duration exposures to HCl gas. + = damage to 1 to 12 plants, o = no damage. Computer derived curves: +1 is one leaf damage, +0 is no leaf damage.

Table 15. Response of short-term exposures of radishes to different concentrations of HCl gas

HCl Concentration (mg/m^3)	Damage	Exposure time (minutes) 5	10	15	20
1-10	plant				
	leaf				
11-20	plant			23/24[1]	15/24
	leaf			69/144[2]	37/139
21-30	plant	5/48	29/60		
	leaf	10/272	86/325		
31-40	plant	5/24	10/12	20/24	9/12
	leaf	9/129	34/59	77/110	38/62
41-50	plant	3/12	10/12	12/12	
	leaf	9/57	23/61	51/60	

[1]Number of plants with any injury/total number of plants exposed

[2]Number of leaves with any injury/total number of leaves exposed.

Discussion

The threshold data presented here show that approximately 20 mg/m^3 for 20 minutes would be enough to damage any of the plants in the study. Some plants such as radish are more sensitive and would be damaged by as little as 10 mg/m^3 HCl gas. Threshold damage in this context refers to at least 1 in 12 plants being injured and injury might be only one leaf on that plant. Sensitivities of individual plants in a group or of different varieties could result in damage at much lower concentrations of HCl, but probably not below 5 mg/m^3 for 20 minutes.

E. Investigating the phytotoxic effect of aluminum oxide plus HCl

Methods

In order to establish whether aluminum oxide has any phytotoxic effect, two general investigations were undertaken. In one, plants were exposed to single fumigations of aluminum oxide plus HCl (1:1) or HCl alone. Damage was recorded after 24 hours and the plants were then discarded. In the other study, plants were exposed to the toxicant combinations once a week for four consecutive weeks. Visual plant injury was noted 24 hours after each exposure. One week after the final fumigation, the plants were harvested and the tops and roots were oven-dried and weighed. This cumulative experiment, therefore, bases phytotoxic response on something besides visual observations.

In the single exposure experiment, marigold plants 30 to 40 days old and with six leaf sets were exposed to HCl gas alone or with aluminum oxide particles. The concentrations ranged from less than 10 to nearly 40 mg/m^3 HCl with a 1:1 ratio of HCl to aluminum oxide when it was used. The plexiglas aerosol chamber can handle 16 plants at a time.

A few single exposures were made on pinto bean plants with the first expanded leaves or with the first trifoliate exposed.

For the cumulative exposures, marigold plants with six leaf sets were exposed to 20-minute doses of HCl or HCl plus aluminum oxide. HCl concentration was either above or near threshold.

Results

Figure 26 shows the response of the marigolds to single exposures of HCl with or without aluminum oxide. A linear correlation was run on the data and differences were seen in the slopes. The slope of the line for exposures with aluminum oxide, 0.694 ($r = 0.968$), was slightly higher than the slope of the line for HCl exposures alone, 0.618 ($r = 0.929$). It is thought that the data may better fit a curvilinear regression analysis and this will be attempted.

Only four groups of beans were fumigated, one with the first trifoliate leaf set fully expanded and the other with only the primary leaves developed. Both leaf sets were quite sensitive to HCl damage with or without aluminum oxide; however, the trifoliate leaf set seemed to show greater differences to the mixed toxicants. This will be repeated.

The two cumulative experiments are summarized in Table 16 and in Figure 27. In the first experiment, lower concentrations of toxicants were used than in the second experiment. Although no visible injury was noted on any plants exposed to about 9 mg/m^3 HCl, at or just below threshold concentration, significant differences were observed in the final dry weights. There was even more reduction in the weight of plants exposed to concentrations which produced some injury on the plants, ca. 18 mg/m^3 HCl.

In the second experiment, the toxicant concentrations were increased to ca. 16 mg/m^3 HCl for threshold and to ca. 26 mg/m^3 for above-threshold, a dose at which all plants sustained visible injury. In this experiment, however, there was an inadvertent increase in the amount of supplemental nutrients that the plants received during part of their growth. From experiments described earlier in this report, this could cause tolerance to the HCl gas. Although the dry weights were again lower than the control, the order was not predictable. Even if the nutrition is not taken into account, this experiment indicated that there is little if any difference when aluminum oxide made up part of the pollutant.

Table 16. Cumulative fumigation of marigold plants

Wk	Treatment	Control	Threshold				Above-Threshold			
			HCl		$HCl+Al_2O_3$		HCl		$HCl+Al_2O_3$	
			Conc.	Damage	Conc.	Damage	Conc.	Damage	Conc.	Damage
			Experiment 1							
1	Fum. 1[1]	--	8.4[2]	0/16[3]	8.8	0/16	11.8	9/16	11.9	12/16
2	Fum. 2	--	9.2	0/16	8.7	0/16	16.7	11/16	17.3	12/16
3	Fum. 3	--	8.5	0/16	8.4	0/16	13.6	11/16	13.1	12/16
4	Fum. 2	--	9.4	0/16	9.3	0/16	30.2	11/16	30.4	12/16
5	Harvest	6.29	5.93		5.06		4.78		3.86	
			Experiment 2							
1	Fum. 1	--	19.8	3/16	18.9	4/16	28.8	16/16	27.5	16/16
2	Fum. 2	--	12.9	7/16	14.4	9/16	24.8	16/16	24.2	16/16
3	Fum. 3	--	15.0	9/16	14.8	13/16	28.5	16/16	25.4	16/16
4	Fum. 4	--	14.1	9/16	14.0	13/16	26.2	16/16	25.3	16/16
5	Harvest	6.47[4]	6.43		6.20		4.81		5.42	

[1]Fum. 1 = first fumigation

[2]HCl concentration in mg/m^3; when aluminum oxide is used, it is approximately equal to HCl concentration.

[3]Number of plants with any damage over total number of plants fumigated.

[4]Mean total dry weight per plant, in grams.

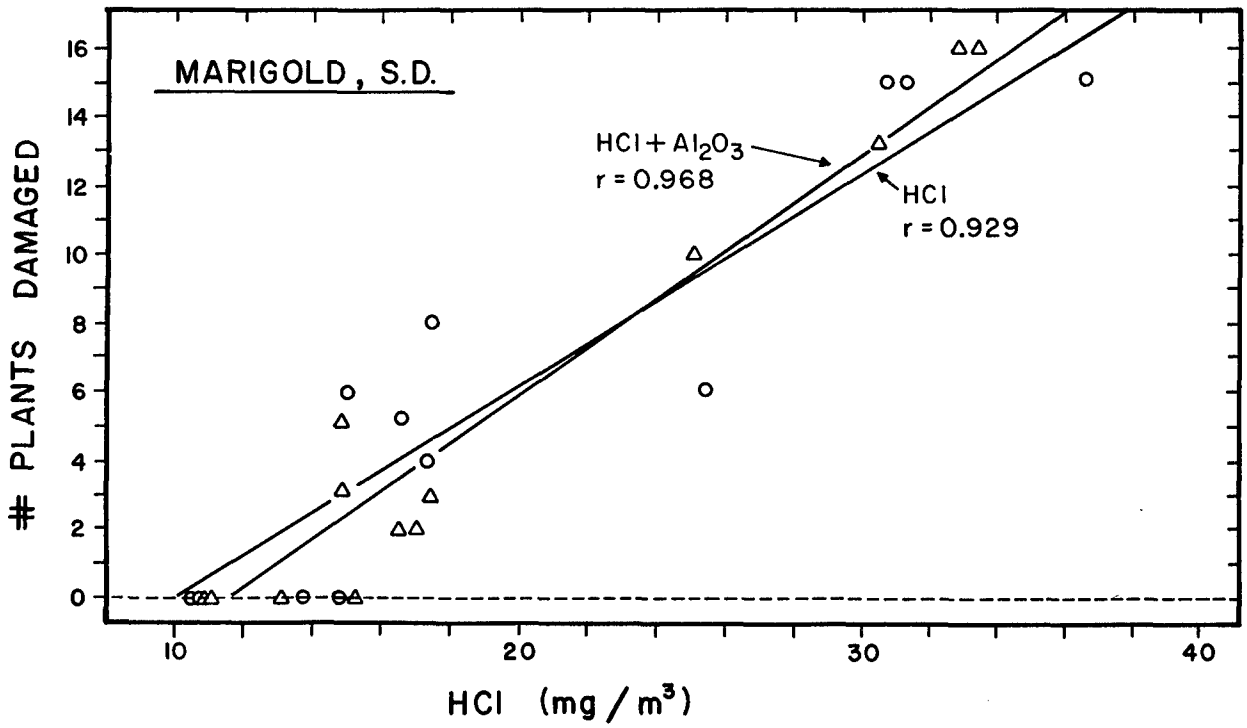


Figure 26. Response of 30- to 40-day-old marigold plants to 20-minute exposures of HCl gas alone (o), or of HCl plus aluminum oxide particles (Δ).

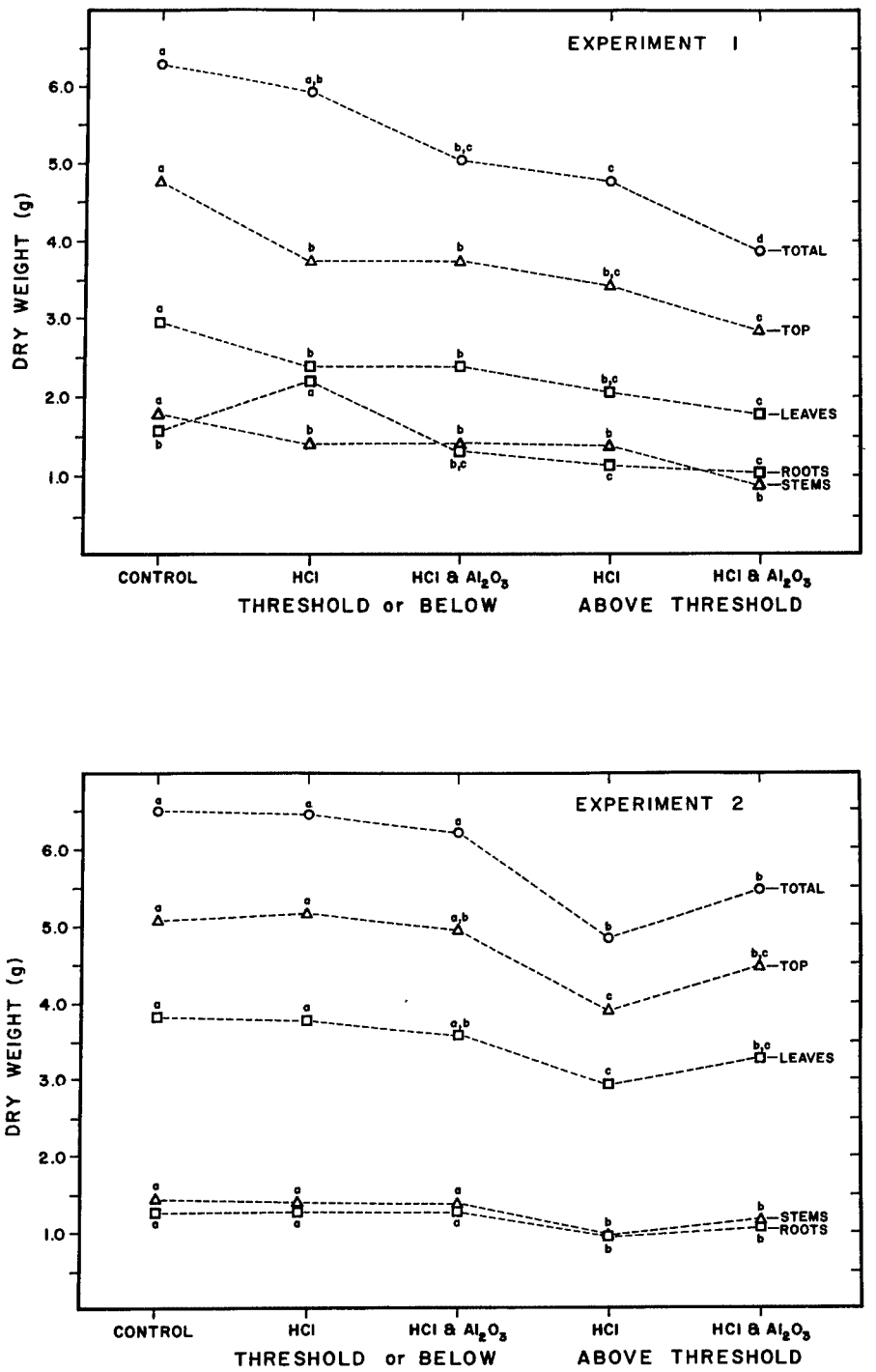


Figure 27. Cumulative effect on the dry weights of parts of marigold plants after four weekly fumigations. Top: Experiment 1, Bottom: Experiment 2.

Discussion

No strong conclusions can be made regarding the injurious effects of aluminum oxide particulates on plants from these experiments. There is evidence from the cumulative experiments that dry weight decreases are found in plants exposed to aluminum oxide in addition to HCl. The preliminary work with the sensitive pinto bean plants indicates an increase in visible injury when exposed to the combined toxicants.

The cumulative experiments indicate that damage to other parts of the plant can certainly occur even though there is no visible damage.

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